Doctoral Thesis

Effects of Long-Term Selection for Non-Destructive Deformation in White Leghorns

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Summary

The improvement of eggshell strength has been an essential research question for several decades, and in spite of the progress made by the poultry industry during the 20th century eggshell quality is expected to remain a major area of study for the years to come. This tendency is not surprising given the projected rapid growth in global egg demand, and in light of the fact that the deficiencies related to poor eggshell quality not only result in substantial economic losses for the producers, but also pose a very serious threat to food safety.

To tackle this problem, researchers have spent years experimenting with a number of approaches to evaluate eggshell strength, analyzing whether or not these methods are efficient, reliable, and practical from an industrial standpoint. Given the nature of the task, however, most of these approaches are inevitably destructive, such as the ones that rely on determining the weight of the eggshell or its thickness. Despite this, non-destructive methods have also been developed. This is the case for instance of non-destructive deformation, whose goal is to assess the strength of the eggshell by measuring its deformation under a standard load. Nevertheless, as of today little research is documented that evaluates the long-term effects of selection for non-destructive deformation.

In this context, the main objective of this doctoral dissertation is to investigate, from different angles, the long-term effects of selection for non-destructive deformation on a variety of phenotypic traits. To this end, particular attention was paid to the analysis of genetic parameters for traits related to eggshell strength, egg production, and egg shape.

The data used for analysis in this thesis was gathered at the National Institute of Livestock and Grassland Science (Tsukuba, Japan) from a selection experiment conducted in White Leghorns over thirty-one generations. In practice, the individuals were divergently selected into two lines based on low and high non-destructive deformation value, referred to as the strong line and the weak line, respectively.
The analysis of the genetic parameters, e.g. genetic correlations and heritabilities, was performed by applying the REML (Restricted Maximum Likelihood) approach in the R computing environment, and the breeding values were estimated by using BLUP (Best Linear Unbiased Prediction) methodology under multivariate animal models.

Several important results were obtained from this experiment. Firstly, the selection process successfully created two clearly distinct lines, providing evidence for the effectiveness of using non-destructive deformation as a criterion for long-term selection. Furthermore, the moderately high heritability values estimated for non-destructive deformation indicate the potential for genetic improvement through selection for non-destructive deformation. It was also established that non-destructive deformation could replace destructive methods to evaluate eggshell strength, for instance eggshell breaking strength, given the high genetic correlations between non-destructive deformation and the other eggshell traits.

Secondly, this experiment revealed a small negative impact from selection for non-destructive deformation on egg production and sexual maturity in the strong line, suggesting the need to balance the selection for non-destructive deformation with that of production traits. At the same time, it was discovered that large egg size (or weight) is not associated with poor eggshell quality.

Thirdly, rounder eggs tended to be somewhat sturdier than more elongated eggs for the range of values studied. This finding is important given that the traits related to the geometry of the egg, e.g. egg shape index, account for a substantial part of the variability in eggshell strength that remains after conventional factors, such as eggshell thickness and specific gravity, have been taken into account.

In consideration of these results and the variety of perspectives addressed in this analysis, it was concluded that non-destructive deformation is a reliable and useful tool that can replace the destructive methods currently used to assess eggshell quality.

Keywords: Egg production, Egg shape, Eggshell strength, Long-term selection, Non-destructive deformation, Poultry, White Leghorn.

Publications

This thesis is based on the following three research articles.

Research Article I

Gervais, O., Nirasawa, K., Vincenot, C., Nagamine, Y., & Moriya, K. (2016). Multiple-trait analysis of a long-term selection experiment for non-destructive deformation in White Leghorns: Evolution of genetic parameters for traits related to eggshell strength. *Livestock Science 189,* 56-62.
doi: 10.1016/j.livsci.2016.05.007

Research Article II

Gervais, O., Nirasawa, K., Vincenot, C., Nagamine, Y., & Moriya, K. (2016). Long-term selection using a single trait criterion, non-destructive deformation, in White Leghorns: Effect over time on genetic parameters for traits related to egg production. *Animal Science Journal xx,* xx-xx.
doi: 10.1111/asj.12653

Research Article III

Gervais, O., Nirasawa, K., Vincenot, C., Nagamine, Y., & Moriya, K. (2016). Effect of long-term selection for non-destructive deformation on egg shape in White Leghorns. *Journal of Poultry Science 53*(4), xx-xx.
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Table of Contents

List of Tables

List of Figures

Chapter I: Introduction

1.1) Domestication and development of chicken breeds

At its beginnings, the history of domestication of chickens, which started with chickens of Indian origin, had little to do with egg and meat production, as fowls were raised back then primarily for cockfighting purposes throughout Asia, Africa, and Europe (Encyclopædia Britannica, 2014); however, it is no mystery that the breeds of chickens which are raised in this day and age for the needs of human consumption have evolved with respect to phenotypic traits, such as body size or egg production, in order to best meet the ever-increasing global demand and the conditions of the world's markets. Despite this, and even though it is largely recognized in the field of animal science that the history of the domestication of chickens dates back several thousand years (West & Zhou, 1989; Nozawa, 1986), it is usually not known of the public that the domestic fowls which are raised today for meat and egg production are considered to have a common ancestor, the Red Jungle Fowl *Gallus gallus* (West & Zhou, 1989; Siegel *et al.*, 1992; Yamashita *et al.*, 1994). Interestingly enough, however, this wild ancestor now differs significantly from its domesticated descendant *Gallus gallus domesticus* with regard to a variety of phenotypic traits of major significance to humans, because of the long domestication process which has taken place all around the world.

To give a few examples of such traits, some of the most distinctive phenotypic differences which have been identified in the domestic fowl in comparison with the Red Jungle Fowl include: plumage color, increase in body size (twofold or more), changes in the size of organs and other body parts (e.g. overall decrease in brain size), earlier onset of sexual maturity, increase in egg weight and egg production, high feed efficiency, rapid growth, as well as changes in behavior (Jackson & Diamond, 1996; Andersson *et al.*, 2000; Schütz & Jensen, 2001; Schütz *et al.*, 2001; Jensen, 2006).

Although a change in most of these traits can be expected given the fact that domestic breeds are typically selected for egg and meat production, the sheer magnitude of the genetic evolution which has occurred throughout the domestication process is compelling. The extent of this development is illustrated for instance by the difference between the egg production of the White Leghorn, which is an efficient egg-laying breed usually selected for its ability to lay a maximum of eggs for a minimum food intake, and that of its wild ancestor, the Red Jungle Fowl. According to a study conducted by Kerje *et al.* (2003), the average egg weight was found to be approximately 23 g for Red Jungle Fowls, for a total egg weight of 97.3 g per week, translating into an average of 4.23 eggs per week.

In comparison, White Leghorns would lay eggs of 57.5 g on average, for a weekly total of 367.1 g (6.38 eggs per week on average). Not only does that study reveal a 150% increase in egg weight for White Leghorns, it shows at the same time an increase in egg production by more than 50%, even after accounting for the increase in egg weight. Such figures illustrate very well the extent to which the domestication process of chicken breeds has influenced their genetic structure, and implicitly raise questions of critical importance for the future of poultry farming, notably with respect to the theoretical limits to this genetic evolution.

1.2) Aim of this dissertation

In the context of this evolution of the genetic structure of chicken breeds, the main goal of this dissertation is to examine and precisely quantify the effects of long-term selection for eggshell strength in White Leghorns. Naturally, eggshell strength has been abundantly studied in the past given that it is an essential attribute for the poultry industry. Nevertheless, surprisingly little research is documented that addresses the long-term effects of selection for non-destructive deformation (Nirasawa *et al.*, 1998).

The present study aims to fill this gap and bring new knowledge to the field by thoroughly analyzing a two-way selection experiment for non-destruction deformation that has been conducted for several decades at the National Institute of Livestock and Grassland Science (Tsukuba, Japan). In particular, this research evaluates the effects of single-trait selection for non-destructive deformation on the three following aspects:

- Traits related to eggshell strength (Chapter IV, Research Article I)
- Traits related to egg production (Chapter V, Research Article II)
- Traits related to egg shape (Chapter VI, Research Article III)

While details about the experiment, the models, and the methods used are provided in Chapter II, it is necessary to state that the scope of this dissertation is limited to the three points above, and will therefore not address other aspects of egg quality, such as issues related to the internal quality of the egg.

Given that this dissertation is meant to be useful, not only for academic research purposes but also for the future development of breeding programs, the core of the analysis will focus on estimating genetic parameters (e.g. variance components and heritabilities) and breeding values, and on examining their evolution over time. To this end, widely established methods will be applied, such as

Restricted Maximum Likelihood (REML; Patterson & Thompson, 1971) and Best Linear Unbiased Prediction (BLUP; Henderson, 1975a). These approaches allow the use of pedigree information and fit the purposes of this thesis as they account for genetic trends and selection, and because it is possible to correct for environmental deviations by adding fixed effects to the model.

As an introduction to these methods, the next section of this opening chapter provides a brief background of the field of animal breeding and genetics. However, rather than delving into the theoretical details of the models used throughout this dissertation (which will be fully explained in Chapter II), stress will be laid on the evolution of the methodology since the 19th century. This chapter will then be concluded by a short discussion of the social contribution of this dissertation.

After the chapter devoted to the methods and model theory, Chapter III will present a basic statistical analysis of the experiment reviewed in this dissertation. This step is essential, not only to gain a broad understanding of the general outcomes of the experiment, but also to be able to understand the subsequent analysis of the genetic parameters and breeding values from the three perspectives covered in the present study (Chapters IV, V, and VI).

Lastly, as the concluding chapter of this dissertation, Chapter VII will summarize the major conclusions of this dissertation and include a brief discussion of the main implications of these findings for the poultry industry.

1.3) A brief history of animal breeding and genetics

The field of animal breeding and genetics as it is generally considered today has a relatively short history. Setting aside traditional methods used around the 17th and 18th century, it appears that little scientific advancement was made before the second half of the 19th century and the publication of *On the Origin of Species* by Charles Darwin (1859), which explains natural selection and lays the foundations of evolutionary biology. It was also then that Gregor Mendel published his works on hybridization, *Experiments on Plant Hybridization* (1866), which, albeit eclipsed by Darwin's theory of evolution, were rediscovered in the early 1900s, most prominently by Correns (1900), de Vries (1900), and Tschermak (1900), thereby giving birth to the so-called Mendelian laws of inheritance.

Because of these advances, tremendous progress was made during the 20th century, which most notably saw the emergence of powerful methods and models for the estimation of variance components and the prediction of random effects like breeding values. During the first part of the 20th century, the research of Jay L. Lush and Sewall Wright, who had developed path coefficient and selection index methods (Wright, 1921, 1934), constituted a solid foundation for the implementation of breeding programs, alongside the fixed effect linear models established by Ronald A. Fisher and Frank Yates; these works were utilized by animal breeders around the late 1930s and early 1940s (Van Vleck, 1998).

However, what stands out the most throughout this evolution of the field of animal breeding and genetics is assuredly the contributions made around the middle of the century (1950s~1970s) by Charles Roy Henderson, who has been unequivocally recognized by all prominent researchers in the field, such as Kennedy (1991), Searle (1991), or Freeman (1991); the many honors and distinctions that he was awarded throughout his life, such as the Borden Award (in 1964), the J.L. Lush Award (in 1982), and the Morrison Award (in 1971) (American Dairy Science Association, 2014; American Society of Animal Science, 2014), as well as his election to the American National Academy of Sciences in 1985 (National Academy of Sciences, 2014), bear witness of his influence in the field. Henderson was even referred to as “the leading world authority” by the Journal of Dairy Science (1989).

Building on the achievements of the first half of the 20th century mentioned above, but using a different approach, Henderson actually managed during the following decades to establish a single model for the analysis of fixed and random effects, which is nowadays more commonly known as Henderson’s Mixed Model Equations (MMEs) (Gilmour, 2009)[1]. Although as aforementioned methods already existed to estimate fixed effects, this result is of essential importance since animal breeders are typically interested in the prediction of breeding values and other genetic parameters (i.e. random effects) rather than in the fixed effects for the development of breeding plans (Van Vleck, 1998); no one before Henderson had been able to find a simple but powerful and computationally feasible method to estimate the BLUP.

As a matter of fact, as described for instance by Van Vleck (Ibid.), before Henderson discovered the MMEs, and given the following general model

$$y = X\beta + \in \tag{1.1}$$

where

y is a vector of observations

β is a vector of fixed effects

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[1] The main point of the MMEs is to efficiently and accurately calculate the Best Linear Unbiased Estimator (BLUE), for fixed effects, and determine at the same time the Best Linear Unbiased Predictor (BLUP), for random effects.

X is the design matrix for the fixed effects, and
$\in$ is a vector of random effects ($E(\in) = 0$),

finding a BLUE solution for β required solving

$$(X'V^{-1}X)\hat{\beta} = X'V^{-1}y \tag{1.2}$$

which in turn required calculating V^{-1}, the inverse of the variance of the observation vector y, defined as $V = Var(y) = Var(\in)$. This was in practice not possible because the correlations among the elements of $\in$ (which would not otherwise exist had there been no random effects) give V a non-trivial structure (i.e. V is non-diagonal), thereby making it too demanding computationally to calculate its inverse.

However, Henderson, Kempthorne, Searle, and von Krosigk (1959) were able to prove that, for the model

$$y = X\beta + Zu + e \tag{1.3}$$

where
u is a vector of random effects
Z is the design matrix for the random effects, and
e is the vector of model residual ($E(e) = 0$)

which, in other words, is the same model as defined in equation (1.1) with $\in = Zu + e$, the matrix V^{-1} could be calculated according to equation (1.4) below,

$$V^{-1} = R^{-1} - R^{-1}Z(Z'R^{-1}Z + G^{-1})^{-1}Z'R^{-1} \tag{1.4}$$

which in simpler terms is equivalent to $V = ZGZ' + R$, where
$R = Var(e)$ (since the elements of e have equal variance and are not mutually correlated, R is a diagonal matrix with all of its elements equal)
G is the variance-covariance matrix for the random effects u, and
$Cov(u, e') = 0$.

These findings by Henderson and his colleagues have radically changed the way animal breeders perform the evaluation of genetic parameters, because the structures of the variance-covariance matrices R^{-1} and G^{-1}make them relatively easy to compute and solve, even for a very large number of equations. In other words, what had seemed practically impossible to solve before the discoveries made by Henderson had suddenly turned into a fairly simple set of equations; the MMEs have allowed animal breeders to assess breeding values accurately (find the BLUP), and have therefore empowered them to develop selection and breeding methods based on efficient and reliable statistical information, even with unbalanced data or with missing records. This explains the success of BLUP and why it quickly became extensively used by animal breeders (and still is today)[2]. In this manner, Henderson's MMEs have become the foundation for mixed model analysis, and, as discussed in the second chapter of this dissertation, have led to the development of complex methods for variance component estimation –namely Maximum Likelihood (ML) and Restricted Maximum Likelihood (REML). This clearly shows the extent and impact of Henderson's work and results on the following decades of research in animal breeding and genetics.

Furthermore, a number of substantial improvements to the MMEs were made in the following years; one example of this is the discovery of efficient and practical ways to compute the numerator relationship matrix (also known as the "A matrix") and its inverse (Henderson, 1976), thereby effectively incorporating pedigree data easily into the MMEs to take into account records from close relatives. Another example of this is the design of the Restricted Maximum Likelihood (REML) procedure to estimate the variance parameters assumed to be known in the MMEs (Patterson & Thompson, 1971). On a slightly different note, it is important to mention that alongside these improvements made on the statistical models used for data analysis, the rapid advances of computer technology which have taken place since the 1980s have not only enabled researchers and animal breeders to implement more computationally demanding procedures (e.g. analyses of larger data sets), they have also created the ideal conditions for the animal model to spread around the world (Sigurdsson, Banos & Philipsson, 1992).

Beside the MMEs and BLUP, a more recent development has seen the appearance of new techniques for genetic selection in animal breeding, notably quantitative trait loci (QTL) experiments and scans, whose use has expanded dramatically since 1994, after the discovery by Andersson *et al*. (1994) of the first QTL for fat deposition in pigs (Rothschild, Hu, & Jiang, 2007). Although the techniques used at the time allowed the detection of some QTL, Meuwissen, Hayes, and Goddard managed to create a simulation showing the potential of genome-wide, high-density mapping procedures to predict the total genetic value (2001). Such results were also later supported by Schaeffer (2006), who is said to be one of the successors of Henderson (Nagamine, 2011), thereby increasing the worldwide acceptance and acknowledgement of high-density SNP genotyping, and pushing for the

[2] As a side note, although it was Henderson who discovered the BLUP, it appears that the term "BLUP" itself was coined by Goldberger (1962).

extensive application of genomic selection. Nevertheless, in spite of the potential of these methods and the higher accuracy that they provide, many studies, such as Visscher *et al.* (2007), highlight that explaining genetic values from the information extracted from high-density SNP genotyping is a complex task. This may explain why most breeding programs still rely on mixed model methodology to predict breeding values (Dekkers, 2012).

1.4) The economic impact of poor egg quality

Before delving into the chapter of this dissertation devoted to clarifying model theory and the methods used for analysis, it is necessary to take a moment to examine how the results achieved through this study concretely –albeit modestly– contribute to the long-term improvement of the breeding practices of our societies. As aforementioned, this thesis focuses predominantly on the evaluation of breeding values and genetic parameters in the White Leghorn, which is an efficient egg-laying breed, in order to improve knowledge and understanding of issues related to the development of breeding programs, and egg quality in general. Since these elements are directly linked to factors such as egg production and egg loss (which is among others a result of poor egg shell quality), it therefore ensues that one of the most evident and logical ways to interpret the results obtained in this paper is from an economic perspective.

In order to better understand the scale of the industry as well as the significance and impact of egg quality on the economy, it is first necessary to provide a brief overview of the current situation, in simple terms. In some of the world’s most powerful economies it can be summarized as follows.

In the United States of America, approximately 83.66 billion eggs for consumption were produced in 2013 (USDA, 2014), which, at an estimated average price of $1.247 per dozen (Mathews & Haley, 2014), represents a yearly revenue in the region of $8.69 billion. In Japan, the Ministry of Agriculture, Forestry and Fisheries estimated the gross value of output for eggs to amount to about ¥441.9 billion[3] for the year 2010 (Ministry of Agriculture, Forestry and Fisheries, 2012). Similarly, the production of eggs for consumption in the European Union[4] in 2013 reached 6.521 million tons (European Commission, 2014a), which translates into a revenue of approximately €8.42 billion[5] for an average price calculated for that year to be about €129.05 per 100 kg (European Commission, 2014b).

[3] Approximately $4 billion (for an exchange rate of $1 = ¥110).

[4] In this case, the figures include data from only 27 member states, as Croatia joined the European Union on July 1, 2013. The European Union comprises 28 member states as of 2014.

[5] Approximately $11 billion (for an exchange rate of $1 = €0.765).

With this in mind, it seems relevant to ask what the financial costs owing to poor egg quality actually are. Many researchers have already tried to answer this complex question, and although the exact percentage of the egg production lost because of poor egg quality is difficult to assess, it seems clear that this represents a significant amount. For instance, some studies have calculated that poor eggshell quality made about 6% of the production uncollectible (Roland, 1977), while other estimated that before reaching the consumer, egg loss due to poor eggshell quality could amount to 13% of the eggs produced (Roland, 1988). Moreover, the problems related to eggshell quality, such as shell breakage, which is an element of central element of concern in this dissertation, have been reported to account for about 80% to 90% of egg deficiencies (Pavlovski *et al.*, 2012).
In broad terms, and based on the figures described above for some of the world's biggest markets, this 6%~13% percentage range is roughly equivalent to a loss of between $521 million and $1.13 billion in North America, between €505 million and €1.09 billion[6] in Europe, and between ¥26.5 billion and ¥57.5 billion[7] in Japan for the corresponding years. On top of this cost, even for eggs which make it to the consumers, it often happens that poor eggshell quality leads to downgrading (Hunton, 1982). In practice, this means that producers incur an additional loss of income given that the retail price of eggs increases with grade (Perez, Weimar, & Cromer, 1991). In any case, regardless of the exact sum such losses amount to, Bell reminds us of a simple but clear fact, "the significant economic impact of 1% egg breakage on overall profits in the egg industry" (1998).

Furthermore, from a different perspective, the Food and Agriculture Organization expects global demand to continue to increase at a fast pace throughout the 21st century, in particular in industrializing countries located in Sub-Saharan Africa and South Asia; for example, for the period from 1990 to 2050, it is estimated that egg demand in these two regions will increase by factors of more than ten and eight, respectively (Bouwman, 1997). Even though this general trend can be explained by a combination of global population growth and a rapidly increasing demand per capita, it will be essential for the population of egg-laying hens to grow in all parts of the world to meet these ever-increasing needs.

In light of the tendency described above regarding future global demand for poultry eggs, it appears that the economic aspects associated with egg quality constitute strong arguments for the promotion of research in animal breeding. In this regard, the most substantial contribution of this dissertation in the field lies in providing certain insight into a number of factors affecting egg quality, in particular by improving the understanding of the genetic parameters and breeding values of chicken breeds through selection for eggshell strength.

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[6] Between approximately $660 million and $1.42 billion (for an exchange rate of $1 = €0.765).

[7] Between approximately $240 million and $523 million (for an exchange rate of $1 = ¥110).

Chapter II: Methods and Model Theory

2.1) Data collection and selection methods

The results presented in this dissertation are essentially based on the analysis of a set of 12,422 records of White Leghorns *(Gallus gallus domesticus),* gathered at the National Institute of Livestock and Grassland Science, located in Tsukuba, Ibaraki, Japan. Detailed information about these records is given below (Table 2.1).

In terms of methods, the White Leghorn population underwent a two-way selection process for eggshell strength during the first thirteen generations; in practice, eighty females and ten males were selected from each generation depending on their performance[1] and used as the parent population to produce the following generation. However, given the fact that the total population is constituted of a somewhat small number of individuals per generation (more precisely, this number was progressively reduced from the thirteenth generation on), and to prevent the coefficient of inbreeding from increasing, the following four generations (generations 14 to 17) were selected using a within-family selection procedure, i.e. choosing a single –but genetically superior– individual per family. From generation 18 onward, individuals were randomly chosen and mated within their lines. In other words, selection was somewhat relaxed after generation 14 and suspended at generation 18. Nevertheless, the lines were both maintained until generation 31.

Overall, there were therefore thirty-one generations of White Leghorns which were divergently selected into two lines based on low and high non-destructive deformation values, referred to as the strong line and the weak line, respectively. There were in total 6,519 records for the strong line and 5,903 records for the weak line, as indicated in Table 2.1. Non-destructive deformation was used as the only selection criterion for all of the generations except the first one, for which breaking strength had to be used instead due to technical difficulties.

On a complementary note, it is important to mention that the base population –corresponding to generation 0– was only used for pedigree information and that the first generation, which is composed of 427 individuals, was therefore used to initiate the two-way selection process and is as a consequence exactly the same for both lines. As a result, each line consists of thirty generations of separate and unique records.

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[1] Males were evaluated on the basis of the full-sib mean given that they do not have individual records.

Table 2.1. Description of the number of individuals and records per generation.

	Weak line				Strong line			
Generation	Females	Males	Total	Records	Females	Males	Total	Records
1	412	15	427	412	412	15	427	412
2	300	8	308	300	284	11	295	284
3	268	10	278	268	275	10	285	275
4	258	11	269	258	266	10	276	266
5	280	10	290	280	301	10	311	301
6	291	11	302	291	297	11	308	297
7	271	10	281	271	290	10	300	290
8	265	10	275	265	288	10	298	288
9	283	10	293	283	292	9	301	292
10	277	10	287	277	288	10	298	288
11	250	10	260	250	248	10	258	248
12	250	67	317	250	254	68	322	254
13	187	70	257	187	198	77	275	198
14	136	69	205	136	130	76	206	130
15	129	67	196	129	136	76	212	136
16	140	68	208	140	146	74	220	146
17	97	34	131	97	150	69	219	150
18	91	23	114	91	135	56	191	135
19	132	65	197	132	146	63	209	146
20	82	52	134	82	118	66	184	118
21	121	72	193	121	123	113	236	123
22	66	139	205	66	114	234	348	114
23	175	75	250	175	250	86	336	250
24	185	90	275	185	218	96	314	218
25	104	55	159	104	160	81	241	160
26	193	109	302	193	228	73	301	228
27	124	63	187	124	215	100	315	215
28	135	110	245	135	137	101	238	137
29	167	99	266	167	161	84	245	161
30	160	96	256	160	146	92	238	146
31	74	51	125	74	113	77	190	113
Total	5,903	1,589	7,492	5,903	6,519	1,878	8,397	6,519

Each of these individual records consists of a series of measurements regarding the following phenotypic traits: egg weight, non-destructive deformation, eggshell breaking strength, eggshell thickness, eggshell weight, egg production, sexual maturity, egg width, egg length, as well as egg shape index (the egg width to length ratio). Although the measurements for egg production and sexual maturity could only be obtained for the first seventeen generations, the other traits were measured for all of the generations. Non-destructive deformation, as an exception, was measured for all of the generations aside from generation 1. Detailed information and additional notes about the measurements performed are presented in Table 2.2 below.

Table 2.2. Additional information about the phenotypic traits measured.

Trait	Measurement period	Comments
Egg weight	Generation 1 to 31	Measured in g.
Non-destructive deformation	Generation 2 to 31	Measurement of deformation for a load of 1 kg (in µm/kg).
Eggshell breaking strength	Generation 1 to 31	Measured in kg.
Eggshell thickness	Generation 1 to 31	Measured in µm, before drying (see Eggshell weight).
Eggshell weight	Generation 1 to 31	Eggshell weight (including eggshell membrane) in g, after drying for one hour at about 102°C.
Egg width & Egg length	Generation 1 to 31	Measured in cm.
Egg shape index	Generation 1 to 31	Calculated as the egg width to length ratio (in %).
Egg production	Generation 1 to 17	Number of eggs laid per female per 100 days (for hens between 181 and 270 days of age).
Sexual maturity	Generation 1 to 17	Sexual maturity refers to the age of the hen (in days) on the day when she laid her first egg.

While more comprehensive statistics and figures will be provided about this data set in the following chapters, it is important to mention a few more words about the relevance and significance of this dissertation before moving on to the next section.

In the field of animal science, studies based on extremely large sets of data are conducted very frequently, most prominently in relation to dairy cattle (notably Holstein-Friesian cattle), with data sets often containing hundreds of thousands –if not millions– of records, such as in Misztal *et al.* (1993), Hansen *et al.* (2004), and Oseni *et al.* (2003). Likewise, in the case of poultry farming, it is not uncommon to find studies where divergent selection is used over several generations, among others for the purpose of multiple-trait analysis; this is for example the case in Mielenz *et al.* (1994), Anang *et al.* (2000), and Sharma *et al.* (1996), who analyzed data sets gathered from White Leghorn populations over seven, nine, and sixteen generations, respectively. Studies also exist that were conducted over even longer periods of time –tens of generations–, such as Dunnington and Siegel (1996), who analyzed an experiment carried out on divergently selected White Plymouth Rock chickens over thirty-eight generations.

At the same time, it can be seen quite clearly from Hunton's overview of poultry genetics (2006) that much of the research conducted in poultry science focuses specifically on egg production, as illustrated by the studies mentioned above; this is not surprising given the fact that one of the main objectives of research related to animal breeding is to improve breeding plans, and that egg production is an essential selection criterion for commercial poultry breeders.

Nevertheless, although this by no means implies that only little attention has been paid to issues related to the structure of eggshells, to eggshell strength, eggshell breakage, or non-destructive deformation –as numerous studies address the matter (Pevzner, Friars, Orr, & Reinhart, 1976; Hunt, Voisey, & Thompson, 1977; Hamilton, Thompson, & Voisey, 1979; Voisey, Hamilton, & Thompson, 1979)–, to the knowledge of the author, little research is documented on long-term selection for eggshell strength through non-destructive deformation, apart from a study by Nirasawa *et al*. (1998) which was conducted on ten generations of White Leghorns. A longer time frame is however needed in order to further investigate the genetic trends displayed in that study, to determine the selection limits with regard to eggshell strength, as well as to provide more accurate breeding values for the phenotypic traits considered: This perspective is what this thesis aims to provide.

2.2) Mixed linear models

In order to comprehend the theoretical concepts introduced in this dissertation, it is necessary to devote an entire section to the development and understanding of mixed linear models, upon which the analyses conducted throughout this paper rely. Although mixed models have been widely explored and are often used in many research areas, it is essential, before proceeding to a deeper analysis of the data set presented above, to clearly define and delimit the scope of the theoretical framework, as well as explain how mixed linear models contribute to the present research.

The first part of this section is devoted to the derivation of the mixed model equations (MMEs) developed by Henderson, and to demonstrating that they indeed yield the BLUE and BLUP, for fixed and random effects, respectively. A few notes also cover some of the advantages of the MMEs over older methods. Parts 2.2.2 and 2.2.3 are needed to give details about how these MMEs can be expanded and further developed to yield a model which is both adequate and practical to accomplish the analyses required to fulfill the aims defined in the first chapter of this dissertation.

2.2.1) The Mixed Model Equations of Henderson

As mentioned in the first chapter, the MMEs discovered by Henderson around the middle of the 20th century represent a practical way to compute the BLUP and therefore accurately predict the genetic values necessary to the development of effective breeding programs. For that reason, the main objective of this section is to explain in detail the mixed model theory on which most of the analysis performed throughout this paper is founded.

To start simply, even the most elementary mixed model which can be used to describe a given phenotypic trait (or observation) in an animal has to include two main variables: the effects related to the environment of the individual, and the effects related to the genes that the individual possesses. In mathematical terms, this is represented by equation (2.1) below,

$$y_{ij} = \mu_i + g_i + e_{ij} \tag{2.1}$$

where

y_{ij} is the j^{th} observation on the i^{th} individual,
μ_i represents the fixed environmental effects of the i^{th} individual,
g_i represents the (random) genetic effects of the i^{th} individual, and
e_{ij} refers to random residual effects affecting the j^{th} observation on the i^{th} individual.

It is important to note that although the fixed environmental effects are generally easily identifiable (this may be for instance the sex, year of birth, or generation of any given individual), the genetic component, g_i, comprises three different values: the additive genetic value, the dominance genetic value, and the epistatic genetic value. As explained for example by Mrode (1996), the additive genetic value refers to the average additive effects of the genes that an individual receives from both parents (in other terms, it is the individual's breeding value), and because it is the only component which can be selected for[2], it is the key factor of interest for the development of any breeding program. In comparison to the additive genetic value, the dominance and epistatic genetic values, which refer to the value of intralocus and interlocus interactions among genes, respectively, are often considered to be negligible, therefore explaining why they are most often included in the model as part of the residual effect term e_{ij} (Ibid.). The present dissertation is also an illustration of this since one of the main points of interest lies in determining the breeding values for thirty-one generations of two-way selected White Leghorns; the only random effect considered was therefore the additive genetic value of each individual.

In matrix form, the model described above in equation (2.1) can be generalized, thereby expanding to a slightly more sophisticated form, as indicated by equation (2.2) below.

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[2] This is because the additive genetic value of an individual is the sum of the average effects of the genes transmitted from both parents, and is thus a function of the genes transmitted from the parents.

$$\begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix} = X \begin{pmatrix} b_1 \\ \vdots \\ b_p \end{pmatrix} + Z \begin{pmatrix} u_1 \\ \vdots \\ u_q \end{pmatrix} + \begin{pmatrix} e_1 \\ e_2 \\ \vdots \\ e_n \end{pmatrix} \tag{2.2}$$

This model can be rewritten more simply as

$$y = Xb + Zu + e \tag{2.3}$$

where

y is a $n \times 1$ vector of observations (n being the total number of records),
b is a $p \times 1$ vector of fixed effects (p being the number of levels for fixed effects),
X is the $n \times p$ incidence matrix for b, relating it to the observation vector y,
u is a $q \times 1$ vector of random effects (q being the number of levels for random effects),
Z is the $n \times q$ incidence matrix for u, relating it to the observation vector y, and
e refers to a vector of random residual effects associated with each individual record.

The model described in equation (2.3) has been widely used (e.g. Searle 1997a) and is usually referred to as a mixed model because of its accounting for both fixed and random effects (Eisenhart, 1947). By definition, the expected values of the variables are $E(e) = 0$ and $E(u) = 0$, which consequently leads to $E(y) = E(Xb + Zu + e) = Xb$. Defining the variance of random effects and random residual effects (which as aforementioned also include non-additive genetic effects) as $var(u) = G$ and $var(e) = R,$ respectively, this logically implies that

$$\begin{aligned} var(y) &= var(Xb + Zu + e) \\ &= var(Zu + e) \\ &= var(Zu) + \ var(e) + cov(Zu, e) + cov(e, Zu) \\ &= Zvar(u)Z' + \ var(e) + Zcov(u, e) + cov(e, u)Z' \end{aligned} \tag{2.4}$$

As already described in the first chapter, this yields, for $var(y) = V$, and under the assumption in this case that $cov(u, e') = 0$ (i.e. u and e are uncorrelated),

$$V = ZGZ' + R \tag{2.5}$$

It is also assumed that the variance matrices G and R are known. With regard to the general model defined through equation (1.1) (i.e. $y = Xb + \in$), it has been known for a long time –from what are known as the “Aitken equations” (Hinkelmann, 1997; Aitken, 1935)– that, if V is non-singular, the normal equations obtained from the generalized least squares procedure are

$$(X'V^{-1}X)\hat{b} = X'V^{-1}y \tag{2.6}$$

with $V = var(\in)$, and with solution

$$\hat{b} = (X'V^{-1}X)^{-}X'V^{-1}y \tag{2.7}$$

From equation (2.7), the generalized least squares estimator (GLSE) of Xb, which is also the BLUE of Xb, is therefore given by

$$BLUE(Xb) = GLSE(Xb) = X\hat{b} = X(X'V^{-1}X)^{-}X'V^{-1}y \tag{2.8}$$

Additionally, from equations (2.5) and (2.6), then $\hat{b}$, the estimator of b, and solution to $y = Xb + Zu + e$, can be obtained from equation (2.9) below

$$X'(ZGZ' + R)^{-1}X\hat{b} = X'(ZGZ' + R)^{-1}y \tag{2.9}$$

Therefore, although it was mentioned in the first chapter that solving the model by inverting V is often not feasible computationally, equation (2.6) should still hold for any attempt to find a BLUE solution to equation (2.3). Also, from the definitions and assumptions made about the model (2.3),

$$\begin{aligned} cov(y,u) &= cov(Zu + e, u) \\ &= cov(Zu, u) + cov(e, u) \\ &= Zcov(u, u) \\ &= ZG \end{aligned} \tag{2.10}$$

From these deductions it is possible to find an estimate $\hat{u}$ for u, which is, as underlined by Searle (1997b), an estimator of the conditional mean of u given y, i.e. $E(u|y)$, from which the following expression can be derived:

$$\begin{aligned} \hat{u} &= E(u|y) = E(u) + cov(u, y')[var(y)]^{-1}[y - E(y)] \\ &= GZ'V^{-1}(y - Xb) \end{aligned} \tag{2.11}$$

Similarly to the fact that equation (2.6) should hold for any attempt to find a BLUE solution to the model described through equation (2.3), equation (2.11) should remain true for any estimate of u to actually be the BLUP of u.

In the development of the MMEs, one of the turning points was achieved when Henderson found a way to calculate what he called at the time the “joint maximum likelihood estimates” of b and u (Henderson, 1950), although he suggested later (Henderson, 1973; Robinson, 1991) that these estimates should not be called so because $\hat{b}$ and $\hat{u}$ are derived by maximizing the joint density function of y and u with respect to b and u, and therefore do not represent a likelihood.

Although it was discovered later that normality is not a requirement for the MMEs to hold true (Robinson, 1991), Henderson's calculations were first made under the assumption that e and u are normally distributed with variance matrices R and G, respectively. In simpler terms, $e \sim N(0, R)$ and $u \sim N(0, G)$. As summarized by Searle (1997b), for this model, the joint density function is

$$\begin{aligned} f(y,u) &= g(y|u)h(u) \qquad (2.12) \\ &= Ce^{\left\{-\frac{1}{2}(y-Xb-Zu)'R^{-1}(y-Xb-Zu)\right\}}e^{\left\{-\frac{1}{2}u'G^{-1}u\right\}} \end{aligned}$$

where C is a constant. Maximizing equation (2.12) is equivalent to maximizing the following expression,

$$\log f(y,u) \propto -\frac{1}{2}\{(y-Xb-Zu)'R^{-1}(y-Xb-Zu)+u'G^{-1}u\} \qquad (2.13)$$

which in turn requires minimizing

$$(y-Xb-Zu)'R^{-1}(y-Xb-Zu)+u'G^{-1}u \qquad (2.14)$$

This can be achieved through differentiation with respect to b and u, leading to expressions (2.15) and (2.16) respectively.

$$\frac{\delta \log f}{\delta b} \propto X'R^{-1}(y-Xb-Zu) \qquad (2.15)$$

$$\frac{\delta \log f}{\delta u} \propto -Z'R^{-1}(y-Xb-Zu)+G^{-1}u \qquad (2.16)$$

Setting these two expressions to zero gives the following set of equations, whose solutions $\tilde{b}$ and $\tilde{u}$ are, as will be demonstrated in the following pages, BLUE and BLUP estimates of b and u, respectively, for the model defined through equation (2.3).

$$X'R^{-1}y = X'R^{-1}X\tilde{b}+X'R^{-1}Z\tilde{u} \qquad (2.17)$$

$$Z'R^{-1}y = Z'R^{-1}X\tilde{b}+(Z'R^{-1}Z+G^{-1})\tilde{u} \qquad (2.18)$$

Although Henderson first presented this set of equations in 1950 in summation form (Henderson, 1950), these mixed model equations have become more popular in the following years in matrix form; rewriting equation (2.17) and (2.18) yields Henderson's MMEs as they are known today:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z+G^{-1} \end{bmatrix} \begin{bmatrix} \tilde{b} \\ \tilde{u} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{bmatrix} \tag{2.19}$$

One advantage of equation (2.17) over equation (2.6) is that the matrix calculations involved are much easier to compute; as a matter of fact, whereas the covariance matrix $V,$ and thus its inverse $V^{-1},$ had dimensionality $n \times n,$ the dimensionality of the matrix on the left-hand side of the MMEs (which needs to be inverted to find a solution for $\tilde{b}$ and $\tilde{u}$) is only $(p+q) \times (p+q),$ which is in most cases far smaller (Henderson, 1973). Another advantage of the MMEs is that they also yield the BLUP along with the BLUE, as it is only one set of equations.

Before moving on to the next step, however, it is important to give the mathematical demonstration that the $\tilde{b}$ estimator found in equation (2.19) actually is the same as $\hat{b}$ in equation (2.7), i.e. that it is the BLUE of $b,$ and that $\tilde{u}$ in (2.19) indeed is the BLUP of u, as indicated in equation (2.11). These proofs, which were first given by Henderson, Kempthorne, Searle, and von Krosigk (1959) and Henderson (1963), can be derived as follows. From equation (2.18) –or from the second equation of (2.19) (bottom part)–, it is possible to rewrite $\tilde{u}$ as

$$\begin{aligned} \tilde{u} &= (Z'R^{-1}Z+G^{-1})^{-1}\left(Z'R^{-1}y - Z'R^{-1}X\tilde{b}\right) \\ &= (Z'R^{-1}Z+G^{-1})^{-1}Z'R^{-1}(y - X\tilde{b}) \end{aligned} \tag{2.20}$$

This equivalence for $\tilde{u},$ when substituted into equation (2.17) –or from the first equation of (2.19) (upper part)–, gives

$$X'R^{-1}X\tilde{b} + X'R^{-1}Z(Z'R^{-1}Z+G^{-1})^{-1}Z'R^{-1}(y - X\tilde{b}) = X'R^{-1}y \tag{2.21}$$

which, for $W = (Z'R^{-1}Z+G^{-1})^{-1}$, yields, as explained by Mrode (1996)

$$X'R^{-1}X\tilde{b} - X'R^{-1}ZWZ'R^{-1}X\tilde{b} = X'R^{-1}y - X'R^{-1}ZWZ'R^{-1}y \tag{2.22}$$

$$X'(R^{-1} - R^{-1}ZWZ'R^{-1})X\tilde{b} = X'(R^{-1} - R^{-1}ZWZ'R^{-1})y \tag{2.23}$$

For $V^{-1} = R^{-1} - R^{-1}ZWZ'R^{-1}$, this is equivalent to

$$X'V^{-1}X\tilde{b} = X'V^{-1}y \tag{2.24}$$

which, as aforementioned, is the generalized least square solution for b. However, in order to demonstrate that the $\tilde{b}$ estimator from the MMEs is a generalized least square (GLS) solution of b it must be proved, to be consistent with the assumptions made so far, i.e. that $V^{-1} = R^{-1} - R^{-1}ZWZ'R^{-1}$ is indeed the inverse of $V = ZGZ' + R,$ as defined in equation (2.5). This was showed by Henderson, Kempthorne, Searle, and von Krosigk (1959), who proved that the identity $(ZGZ' + R)V^{-1} = I$ is true; the proof is given below.

$$\begin{aligned}(ZGZ' + R)V^{-1} &= (ZGZ' + R)(R^{-1} - R^{-1}ZWZ'R^{-1}) \\ &= I + ZGZ'^{R^{-1}} - ZWZ'^{R^{-1}} - ZGZ'^{R^{-1}}ZWZ'^{R^{-1}} \\ &= I + ZGZ'R^{-1} - Z(I + GZ'R^{-1}Z)(Z'R^{-1}Z + G^{-1})^{-1}Z'R^{-1} \\ &= I + ZGZ'R^{-1} - ZG(G^{-1} + Z'R^{-1}Z)(Z'R^{-1}Z + G^{-1})^{-1}Z'R^{-1} \\ &= I + ZGZ'R^{-1} - ZGZ'R^{-1} \\ &= I\end{aligned} \tag{2.25}$$

This proves that $\tilde{b},$ the solution for b obtained from the MMEs is equal to $\hat{b},$ the GLS solution found in equation (2.6). Additionally, as demonstrated by Searle (1997a), from equation (2.20), $\tilde{u}$ from the MMEs is equal to

$$\begin{aligned}\tilde{u} &= (Z'R^{-1}Z + G^{-1})^{-1}Z'R^{-1}(y - X\tilde{b}) \\ &= (Z'R^{-1}Z + G^{-1})^{-1}Z'R^{-1}VV^{-1}(y - X\tilde{b}) \\ &= (Z'R^{-1}Z + G^{-1})^{-1}Z'R^{-1}(ZGZ' + R)V^{-1}(y - X\tilde{b}) \\ &= (Z'R^{-1}Z + G^{-1})^{-1}(Z'R^{-1}ZGZ' + Z')V^{-1}(y - X\tilde{b}) \\ &= (Z'R^{-1}Z + G^{-1})^{-1}(Z'R^{-1}Z + G^{-1})GZ'V^{-1}(y - X\tilde{b}) \\ &= GZ'V^{-1}(y - X\tilde{b})\end{aligned} \tag{2.26}$$

With $\tilde{b}$ being the BLUE of $b,$ this means that $\tilde{u}$ is the same as $\hat{u}$ in equation (2.11), and is therefore the BLUP of u. This shows that the MMEs as defined in (2.19) are an efficient way to calculate the BLUE of b and BLUP of u.

2.2.2) The Numerator Relationship Matrix

The MMEs derived in part 2.2.1 represent the basic form of the BLUE and BLUP solutions for the model $y = XB + Zu + e$; however, depending on the methods used for data collection, and on the data itself, it is possible to adapt them to best fit the analytical needs of the user. One of the valuable findings of Henderson lies in accounting for pedigree information (genetic relationships among individuals) through the insertion in the model of what is called the numerator relationship matrix (NRM), commonly defined as A, or, more precisely, of its inverse, A^{-1}. As a matter of fact, even though the principles behind the A matrix itself are not a discovery attributed to Henderson –Wright for example published studies on how to evaluate coefficients of inbreeding (1922)–, he is the one who initially used the A matrix as a concrete means to use available information about relatives for the estimation of breeding values.

More importantly, Henderson also and above all found practical and efficient ways to directly compute the inverse, which is needed for the model, without calculating and inverting the A matrix itself (Henderson, 1976). This is of the greatest significance, because as will be seen in the coming pages, it is A^{-1}, and not A, which must be known to solve the MMEs, and given the dimensionality of the matrix ($n \times n$), it is as computationally demanding to calculate its inverse as it was to obtain V^{-1} from V (cf. Chapter I, Section 1.3). Since the main analysis of the data set used throughout this dissertation relies almost entirely on pedigree information for the calculation of breeding values, a part of this chapter is devoted to clarifying the assumptions –as well as the actual computations– behind the contribution of the numerator relationship matrix to the model.

The most common case is that of the animal model, i.e. a model which predicts the breeding value (or in other words, the additive genetic effects) of each animal; it is frequently assumed that the variance of residual effects is the same for all individuals and that there is no correlation with any other residual effects. This typical assumption allows the simplification $R = {\sigma_E}^2 I$, where ${\sigma_E}^2$ represents the variance of residual effects, and implies as a direct consequence

$$R^{-1} = {\sigma_E}^{-2} I \tag{2.27}$$

In the animal model, the main interest is the prediction of the breeding values of the individuals, which is a random effect and is therefore represented by vector u in the model. As a consequence, the corresponding covariances can be obtained from the additive genetic covariance between relatives. The numerator relationship matrix A is the matrix which indicates this additive genetic relationship[3], and the individuals appearing in the pedigree (from 1 to n) are ordered in such way that the parents appear before their progeny, starting with the base population[4].

To understand how the A matrix fits into the model, it is necessary to recall that the additive genetic relationship between two individuals is equal to twice their coancestry (Falconer, 1960); as a consequence, this entails that the variance-covariance matrix can be obtained by multiplying this value by ${\sigma_A}^2$, the additive genetic variance in the base population, leading to $G = {\sigma_A}^2 A$. As a direct result of this,

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[3] For this reason, the A matrix is sometimes also called the additive genetic relationship matrix.

[4] The words “base population” generally refer to a population of individuals assumed to be genetically unrelated to each other.

$$G^{-1} = {\sigma_A}^{-2} A^{-1} \tag{2.28}$$

With this in mind, the MMEs of Henderson as presented in equation (2.19) can therefore be rewritten, because of (2.27), as

$$\begin{bmatrix} {\sigma_E}^{-2} X'X & {\sigma_E}^{-2} X'Z \\ {\sigma_E}^{-2} Z'X & {\sigma_E}^{-2} Z'Z + G^{-1} \end{bmatrix} \begin{bmatrix} \tilde{b} \\ \tilde{u} \end{bmatrix} = \begin{bmatrix} {\sigma_E}^{-2} X'y \\ {\sigma_E}^{-2} Z'y \end{bmatrix} \tag{2.29}$$

which then gives, because of equation (2.28)

$$\begin{bmatrix} {\sigma_E}^{-2} X'X & {\sigma_E}^{-2} X'Z \\ {\sigma_E}^{-2} Z'X & {\sigma_E}^{-2} Z'Z + {\sigma_A}^{-2} A^{-1} \end{bmatrix} \begin{bmatrix} \tilde{b} \\ \tilde{u} \end{bmatrix} = \begin{bmatrix} {\sigma_E}^{-2} X'y \\ {\sigma_E}^{-2} Z'y \end{bmatrix} \tag{2.30}$$

Multiplying both sides of (2.30) by ${\sigma_E}^2$ yields

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + {\sigma_E}^2/{\sigma_A}^2 \, A^{-1} \end{bmatrix} \begin{bmatrix} \tilde{b} \\ \tilde{u} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \tag{2.31}$$

which is equivalent to

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + \lambda A^{-1} \end{bmatrix} \begin{bmatrix} \tilde{b} \\ \tilde{u} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \tag{2.32}$$

for $\lambda = {\sigma_E}^2/{\sigma_A}^2 = (1-h^2)/h^2$, with h^2 denoting the heritability. Theoretically, the variances ${\sigma_E}^2$ and ${\sigma_A}^2$ are assumed to be known for the base population, or at least their proportionality, in order to solve the MMEs as presented in equation (2.32). While this is never the case in practice, it is possible to use methods such as restricted maximum likelihood (REML) to estimate them, as described in the following section.

Regarding the actual computation of the inverse of the A matrix, it can be noted that soon after Henderson’s original releases (1975b, 1976), a number of research papers by other authors were published in order to find the best decomposition method and describe possible variants on the model (Thompson, 1977; Quaas, 1976), and much of the subsequent research focused on improving the algorithm to increase computational speed (Hudson, Quaas, & Van Vleck, 1982; Tier, 1990; Meuwissen & Luo, 1992).

Overall, one of the most significant results can be obtained from the decomposition of the A matrix as

$$A = TDT' \tag{2.33}$$

which instantly yields the following expression for A^{-1}, given the fact that T and D are non-singular matrices:

$$A^{-1} = (T^{-1})' D^{-1} T^{-1} \tag{2.34}$$

The advantage of using this decomposition is that the T^{-1} matrix in this equation has a very simple structure; indeed, it is a lower triangular matrix of the order of animals in the pedigree whose diagonal elements are all 1, and with all of the elements to the left of the diagonal being 0 except for those corresponding to the row of animal i and the columns of the known parents, in which case they take the value -0.5. In mathematical terms, defining t_{ij} any element of T^{-1} (with $i, j = 1,2, \dots, n$), this means that $t_{ii} = 1$ and, calling animal d and animal s the dam and the sire of animal i, respectively (with $d < i$ and $s < i$, given the fact that parents appear before their progeny in the matrix, starting with the base population), $t_{id} = t_{is} = -0.5$. This matrix can be further simplified under the form $I - M$, where M is a matrix representing the contribution of gametes from parents to progeny (Kennedy, 1989), with value 0.5 where T^{-1} had -0.5, and 0 otherwise.

Although the calculations involved are more complicated in case of inbreeding, the structure of D^{-1} is also simple, as it is the inverse of the diagonal variance matrix D for Mendelian sampling, whose diagonal elements can be calculated as follows (Mrode, 1996): If both parents of animal i are known,

$$d_{ii} = 0.5 - 0.25(F_d + F_s) \tag{2.35}$$

if only one parent is known (say the dam),

$$d_{ii} = 0.75 - 0.25(F_d) \tag{2.36}$$

and lastly, if no parent of animal i is known, then

$$d_{ii} = 1 \tag{2.37}$$

where F_d and F_s represent the coefficients of inbreeding of the dam and sire, respectively (Wright, 1922).

The major difficulty with these expressions lies in the fact that the calculation of the coefficient of inbreeding for a given individual is influenced by all of its common ancestors, also explaining why the value of d_{ii} in the (2.35), (2.36), and (2.37) reduces to $0.5, 0.75,$ and 1, respectively, when ignoring the effect of inbreeding. While more fine points about the available computation methods can be found for instance in Henderson (1975b, 1976), the present part shows that in spite of the apparent complexity of the A matrix, it is possible to adapt and decompose the MMEs in such a way that practical and computable solutions exist which take full advantage of pedigree information, even for populations with hundreds of thousands or millions of individuals.

2.2.3) The multivariate animal model

One of the assumptions made so far concerning the model described with equation (2.3) is that only a single trait y is recorded and used as a base for analysis to determine the breeding value (or BLUP) of each animal. In the case where records are taken for several traits, although it is possible to perform several single-trait (univariate) analyses to obtain breeding values for each trait, combining the traits into a single model, so called multivariate animal model, gives more accurate values, as originally described by Henderson & Quaas (1976). This gain in accuracy can be explained by the fact that the simultaneous estimation of breeding values for several traits takes into account the (phenotypic and genetic) correlation between these traits. However, one of the main drawbacks of multiple-trait evaluations is the increased computational cost, as well as the need to accurately estimate the (phenotypic and genetic) correlation between the traits[5].

For k traits recorded on a set of n individuals, assuming that a single measurement is recorded per individual per trait, equation (2.3) would become

$$\begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_k \end{pmatrix} = \begin{pmatrix} X_1 & 0 & \cdots & 0 \\ 0 & X_2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & X_k \end{pmatrix} \begin{pmatrix} b_1 \\ b_2 \\ \vdots \\ b_k \end{pmatrix} + \begin{pmatrix} Z_1 & 0 & \cdots & 0 \\ 0 & Z_2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & Z_k \end{pmatrix} \begin{pmatrix} u_1 \\ u_2 \\ \vdots \\ u_k \end{pmatrix} + \begin{pmatrix} e_1 \\ e_2 \\ \vdots \\ e_k \end{pmatrix} \qquad (2.38)$$

where

y_i are $n \times 1$ vectors of observations on the i^{th} trait in the j^{th} individual (with j from 1 to n),

b_i are $p \times 1$ vectors of fixed effects on the i^{th} trait (p being the number of levels for fixed effects),

X_i are $n \times p$ incidence matrices relating b_i vectors to the observation vectors y_i,

u_i are $q \times 1$ vectors of random effects on the i^{th} trait (q being the number of levels for random effects),

Z_i are $n \times q$ incidence matrices relating u_i vectors to the observation vectors y_i, and

e_i refers to the vectors of random residual effects associated with each individual on the i^{th} trait.

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[5] Indeed, if the traits are assumed to be uncorrelated, then the multivariate model is equivalent to performing a univariate analysis for each trait. It is therefore critical to choose traits which are thought (or known) to be correlated.

Many assumptions could be made about this model, but for explanatory purposes it is convenient to assume that there are no missing records and that the incidence matrices X_i and Z_i are equal for all i (that is, $X_1 = X_2 = \cdots = X_k$, and $Z_1 = Z_2 = \cdots = Z_k$). Although the correlation between the k traits for a single individual greatly increases the complexity of the variance-covariance matrices R and G, and therefore the MMEs themselves, for $u' = [u'_1 \quad u'_2 \quad \cdots \quad u'_k]$ and $e' = [e'_1 \quad e'_2 \quad \cdots \quad e'_k]$, these assumptions lead to the following results (Szwaczkowski, 2003):

$$var\begin{bmatrix} u \\ e \end{bmatrix} = \begin{bmatrix} G & 0 \\ 0 & R \end{bmatrix} \tag{2.39}$$

where $R = R_0 \otimes I_n$ and $G = G_0 \otimes A$, in Kronecker product notation. R_0 represents the $k \times k$ variance-covariance matrix for residuals, in other words,

$$R_0 = \begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1k} \\ r_{21} & r_{22} & \cdots & r_{2k} \\ \vdots & \vdots & \ddots & \vdots \\ r_{k1} & r_{k2} & \cdots & r_{kk} \end{bmatrix} \tag{2.40}$$

for r_{ij} referring to the (co)variances between traits i and j. Similarly, G_0 represents the $k \times k$ variance-covariance matrix for additive genetic effects:

$$G_0 = \begin{bmatrix} g_{11} & g_{12} & \cdots & g_{1k} \\ g_{21} & g_{22} & \cdots & g_{2k} \\ \vdots & \vdots & \ddots & \vdots \\ g_{k1} & g_{k2} & \cdots & g_{kk} \end{bmatrix} \tag{2.41}$$

From these equations, expressions for R^{-1} and G^{-1} can immediately be obtained.

$$R^{-1} = {R_0}^{-1} \otimes I_n \tag{2.42}$$

$$G^{-1} = {G_0}^{-1} \otimes A^{-1} \tag{2.43}$$

This is a convenient result, since A^{-1} is already known, and the variance-covariance matrices R_0 and G_0 are only small $k \times k$ matrices whose inverses can be computed instantly, k being the number of traits. Under the assumption that matrices R and G are known, this implies that the MMEs of Henderson (cf. equation (2.19)) become

$$\begin{bmatrix} X'({R_0}^{-1} \otimes I_n)X & X'({R_0}^{-1} \otimes I_n)Z \\ Z'({R_0}^{-1} \otimes I_n)X & Z'({R_0}^{-1} \otimes I_n)Z + {G_0}^{-1} \otimes A^{-1} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} X'({R_0}^{-1} \otimes I_n)y \\ Z'({R_0}^{-1} \otimes I_n)y \end{bmatrix} \tag{2.44}$$

for $X = \begin{pmatrix} X_1 & 0 & \cdots & 0 \\ 0 & X_2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & X_k \end{pmatrix}$.

In a more explicit form, under the assumption made above that X_i and Z_i are equal for all i, and renaming these X_i and Z_i matrices X and Z for convenience purposes for the description below, the leftmost matrix of equation (2.44), which must be inverted to find BLUE and BLUP estimates, can be written as

$$\begin{bmatrix} X'Xr^{11} & X'Xr^{12} & \cdots & X'Xr^{1k} & X'Zr^{11} & X'Zr^{12} & \cdots & X'Zr^{1k} \\ X'Xr^{21} & X'Xr^{22} & \cdots & X'Xr^{2k} & X'Zr^{21} & X'Zr^{22} & \cdots & X'Zr^{2k} \\ \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\ X'Xr^{k1} & X'Xr^{k2} & \cdots & X'Xr^{kk} & X'Zr^{k1} & X'Zr^{k2} & \cdots & X'Zr^{kk} \\ Z'Xr^{11} & Z'Xr^{12} & \cdots & Z'Xr^{1k} & Z'Zr^{11}+A^{-1}g^{11} & Z'Zr^{12}+A^{-1}g^{12} & \cdots & Z'Zr^{1k}+A^{-1}g^{1k} \\ Z'Xr^{21} & Z'Xr^{22} & \cdots & Z'Xr^{2k} & Z'Zr^{21}+A^{-1}g^{21} & Z'Zr^{22}+A^{-1}g^{22} & \cdots & Z'Zr^{2k}+A^{-1}g^{2k} \\ \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\ Z'Xr^{k1} & Z'Xr^{k2} & \cdots & Z'Xr^{kk} & Z'Zr^{k1}+A^{-1}g^{k1} & Z'Zr^{k2}+A^{-1}g^{k2} & \cdots & Z'Zr^{kk}+A^{-1}g^{kk} \end{bmatrix}$$

for r^{ij} elements of ${R_0}^{-1}$ and g^{ij} elements of ${G_0}^{-1}$. Similarly, the right-hand side of the equation can be expressed as

$$\begin{bmatrix} X'r^{11}y_1 + X'r^{12}y_2 + \cdots + X'r^{1k}y_k \\ X'r^{21}y_1 + X'r^{22}y_2 + \cdots + X'r^{2k}y_k \\ \vdots \\ X'r^{k1}y_1 + X'r^{k2}y_2 + \cdots + X'r^{kk}y_k \\ Z'r^{11}y_1 + Z'r^{12}y_2 + \cdots + Z'r^{1k}y_k \\ Z'r^{21}y_1 + Z'r^{22}y_2 + \cdots + Z'r^{2k}y_k \\ \vdots \\ Z'r^{k1}y_1 + Z'r^{k2}y_2 + \cdots + Z'r^{kk}y_k \end{bmatrix}$$

As these explicit forms show, these MMEs quickly become enormous as the number of trait measured increases; as a matter of fact, the dimensionality of the two matrices above are $(qk + nk) \times (qk + nk)$and $(qk + nk) \times 1$, respectively, illustrating why multiple-trait analyses remained for some time practically impossible to perform for a large number of individuals and traits. It is nonetheless possible to use a canonical transformation on the correlated traits in order to create a new vector of uncorrelated variables, from which breeding values can be estimated using single-trait analyses; these variables can then be transformed back into their original form (Lynch & Walsh, 1998; Thompson, 1977; Ducrocq & Besbes, 1993).

2.3) Estimation of variance and genetic parameters

As discussed in the previous section, the MMEs represent a convenient way to compute the BLUE of b and the BLUP of u (i.e. breeding values) for both univariate and multivariate animal models, and are a flexible tool as well, enabling for example to take into account pedigree information. However, one of the underlying assumptions made so far is that the variance-covariance matrices R and G (alternatively, R_0 and G_0) are known. Since it is impossible in practice to determine these values exactly, many research papers have been published which focus on developing workable methods to estimate variance and covariance components as accurately as possible, depending on the many possible assumptions which can be made about the model (Henderson, 1953; Searle, 1968, 1970; Harvey, 1970; Harville, 1977; Schaeffer, Wilton, & Thompson, 1978; Meyer, 1985, 1989). This development can also be explained by the fact that, at the same time, conventional and well-established methods such as ANOVA, which provide unbiased estimates even if the data is not normally distributed, include several requirements which are often problematic in the case of the animal model, such as independence between observations, or balanced sample size; this is a serious issue when working with real sets of records, measured on relatives, and with unbalanced data.

As a potential solution to this issue, Hartley and Rao (1967) first introduced the idea of maximum likelihood (ML) estimation, and a few years later Patterson and Thompson (1971) made improvements on ML to develop what is known as restricted maximum likelihood (REML), a procedure which maximizes the part of the likelihood which does not depend on the fixed effects, thereby removing bias existing with ML (Corbeil & Searle, 1976). This explains why REML has become the preferred method for estimation of variance components. In order to explain clearly the concept of REML, however, it is initially indispensable to understand the mechanism behind ML estimation, as REML is essentially derived from ML.

Recalling that for x_i with mean μ_i and variance ${\sigma_i}^2$, multivariate normal distributions are of the general form

$$p(x) = (2\pi)^{-\frac{n}{2}} \left(\prod_{i=1}^{n} \sigma_i \right)^{-1} exp\left\{ -\sum_{i=1}^{n} \frac{(x_i - \mu_i)^2}{2{\sigma_i}^2} \right\} \tag{2.45}$$

or, in matrix notation,

$$p(x) = (2\pi)^{-\frac{n}{2}} |V|^{-\frac{1}{2}} exp\left\{ -\frac{1}{2}(x-\mu)' V^{-1} (x-\mu) \right\} \tag{2.46}$$

for V being the variance-covariance matrix[6]. As a consequence, for the general mixed model described through equation (2.3), with Xb as mean, the log-likelihood of b and V given the observed data is

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[6] In the case of n independent variables, V is simply a diagonal matrix with $diag(V) = \{{\sigma_1}^2, {\sigma_2}^2, \dots, {\sigma_n}^2\}$.

$$L(b,V|X,y) = -\frac{n}{2}\ln(2\pi) - \frac{1}{2}\ln(|V|) - \frac{1}{2}(y - Xb)'V^{-1}(y - Xb) \tag{2.47}$$

Differentiating with respect to b, the vector of fixed effects, and setting to zero instantly yields expression (2.48)

$$\hat{b} = (X'\hat{V}^{-1}X)^{-1}X'\hat{V}^{-1}y \tag{2.48}$$

which is the BLUE of b found previously. The main point of interest, however, lies in determining the ML estimators for the variance components, which can be obtained similarly, by deriving expression (2.47) with respect to σ_A and σ_E and setting to zero; naturally, these two components are included in V, since $V = ZGZ' + R = \sigma_A ZAZ' + \sigma_E I$, for $G = \sigma_A A$ and $R = \sigma_E I$. This procedure leads to the following ML estimators (for mathematical details, refer to Lynch & Walsh, 1998):

$$tr(\hat{V}^{-1}) = y'\hat{P}\hat{P}y \qquad for \;\; {\sigma_E}^2 \tag{2.49}$$

$$tr(\hat{V}^{-1}ZAZ') = y'\hat{P}ZAZ'\hat{P}y \quad for \;\; {\sigma_A}^2$$

with

$$P = V^{-1} - V^{-1}X(X'V^{-1}X)^{-1}X'V^{-1} \tag{2.50}$$

The main difficulty with this result lies in the fact that finding solutions requires estimating the inverse of the covariance matrix V; since no linear function for these solutions exists, the only way to proceed is to use an iterative procedure.

As aforementioned, the advantage of REML compared to ML is to remove the bias which arises by including the fixed effects. Therefore, based on the ML procedure introduced above, REML estimates can be found by performing a transformation on the model (2.3) in order to obtain a new model without fixed effects, solving to find ML estimates for the new variables, and then transforming these back into the original scale of measurement. These steps lead to the following REML estimators (Lynch & Walsh, 1998)

$$tr(\hat{P}) = y'\hat{P}\hat{P}y \qquad for \;\; {\sigma_E}^2 \tag{2.51}$$

$$tr(\hat{P}ZAZ') = y'\hat{P}ZAZ'\hat{P}y \quad for \;\; {\sigma_A}^2$$

Extension to multivariate models follows logically by using X from (2.38) in (2.47) and replacing V in (2.47) by $V = G + R = G_0 \otimes A + R_0 \otimes I_n$, and by subsequently differentiating with respect to each variance component. Although computationally demanding, several canonical transformations and various iterative procedures have been developed to streamline these calculations (additional technical details can be found in: Schaeffer, 1986; Smith & Graser, 1986; Jensen & Mao, 1988; Meyer, 1991; Searle *et al.*, 1992).

2.4) Programming environment: RStudio and ASReml-R

In parallel with the development of the theoretical models described above, major improvements have been made in computer science, which have made it possible to solve extremely complex systems of equations. These advances have been adopted in a variety of fields, and animal science is no exception. A wide range of statistical tools is now available to researchers to perform data analysis such as multivariate analysis; two of the most prominent examples of such tools, which are widely recognized, and applied in research centers and in the academic sphere, are SAS[7], a statistical piece of software whose development started as early as the 1960s in order to meet the growing needs for analysis of vast amounts of agricultural data (SAS Institute Inc., 2014), and SPSS[8], which was originally developed during the same period for the analysis of social science data, and has since then expanded to many other market segments (SPSS Inc., 2009), before its acquisition by IBM in 2009 (International Business Machines Corporation).

However, the 1990s have also seen the development of R, a language and computing environment whose design is particularly adapted to statistical data analysis. R has drawn much attention since its launch under the terms of the Free Software Foundation’s GNU general license in 1995 (R Core Team, 2014), and is now widely used by students, researchers, statisticians, and engineers all around the world. It was chosen over the other more conventional alternatives as the main tool for data analysis in this dissertation for the following reasons:

- It is an intuitive environment for importing, manipulating, and analyzing data, and for statistical modelling in general;
- There is a wide variety of packages available which are often designed for a specific discipline and make the language very flexible;
- R provides powerful tools for the graphical visualization of data, which is a critical element for researchers, who are expected to present clear and concise reports about the analysis of large and complex data sets;
- Such graphs are easy to customize and export in several different formats;

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[7] SAS stands for “Statistical Analysis System”.

[8] SPSS stands for ”Statistical Package for the Social Sciences”.

- R was originally designed to be a practical and user-friendly environment[9];
- Much potential exists for further expansion in the future as the community and interest grows for R, especially when considering the fact that it has been released only relatively recently (in comparison to SAS for instance);
- It is a free and open-source computing environment, which is a key element for the spreading of science and to ensure that the results obtained are easily reproducible by other researchers;
- R is available on all of the major platforms.

For the above reasons, RStudio, which is an integrated development environment (IDE) for R, appeared to provide the ideal conditions for the computations necessary to the completion of this dissertation. More specifically, because RStudio is made up of four different work areas (a console, a source editor, a workspace, as well as a work area for the management of files, packages, and plots), navigating through the R environment becomes more practical and efficient in comparison with what can be obtained with the original R console only; indeed, RStudio enables the clear organization of data and the concrete visualization of computations, thereby facilitating the programming process and the correction of errors as the project progresses.

Nevertheless, as aforementioned, R is an IDE, and additional packages were used for more advanced calculations and computations; as a matter of fact, since this dissertation deals with complex models and a large amount of data, specific procedures are required for computational efficiency. Even though several conventional packages available for R were used sporadically for the completion of this dissertation, in this case, a more complete package was used for key computations: ASReml-R (Version 3) (Butler, 2009)[10]. This package enables the user to perform multivariate analyses efficiently, to estimate variance components by REML, to handle large amounts of data, as well as to take into account pedigree information. While this may reveal some of the drawbacks of R at this stage, such as the fact that the (free) packages which initially come with the IDE may sometimes appear ill-adapted to performing specific and/or complex analyses, this shows at the same time the flexibility of this IDE and its capacity to adapt to statistical tools which would otherwise not be available.

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[9] The convenience provided by RStudio in particular made it the environment of choice for this dissertation

[10] As a side note, it should be noted that ASReml is an independent piece of software, and that ASReml-R is a version which enables the use of ASReml in an R environment.

Chapter III: Phenotypic Data Analysis

3.1) General information

In the previous chapter, which focused primarily on the methods, models, and theoretical concepts used throughout this thesis, only a few brief and simple paragraphs were devoted to the description of the actual data. By contrast, this chapter consists of a thorough introduction of the data, which is meant to give the readers a general picture of the evolution of the phenotypic traits analyzed. The information presented in this chapter therefore mainly contains basic statistics about the population considered, from means and standard deviations for each generation of White Leghorn, to the population distributions for each trait, to the phenotypic correlations between the traits, to the selection differentials and selection responses for each line.

Although some of these statistics may appear quite rudimentary, the analysis of phenotypic traits presented here does provide essential information; this step is not only necessary to appreciate the effectiveness of the two-way selection process for non-destructive deformation and assess the extent to which this affected the other phenotypic traits, but it is also a prerequisite to understanding the analysis of breeding values and other genetic components presented in later chapters.

Given the fact that the analysis of breeding values using the models detailed in Chapter II was performed separately for each of the two lines and implied the use of pedigree information, the data was organized in the following manner.

One spreadsheet was used per line, one for the strong line and one for the weak line. Since selection took place based on the values measured for non-destructive deformation, the strong line logically corresponds to the line for which non-destructive deformation values were low, and vice-versa for the weak line. Moreover, pedigree information was included in the spreadsheet containing the measurements, by inserting three different columns, one containing the number of the individual to which the measurements corresponds, and two additional columns indicating the numbers of the corresponding dam and sire.

As illustrated by Figure 3.1, the spreadsheets also include information about the line to which the individual belongs (1 for the weak line and 2 for the strong line), the generation number, as well as a number corresponding to the sex of the individual (1 for female and 2 for male). There are several reasons for adding these columns. Firstly, this facilitates counting, for example finding the number of individuals per generation or the number of females. Secondly, it is helpful when handling the data, for example if one wants to organize it in the form of a list of individuals by generation and sex, and thereby helps keep track of the records. Thirdly, this information also simplifies programming procedures.

	A	B	C	D	E	F	G	H	I	J	K	L
1	Ind	Sire	Dam	Line	Gen	Sex	Egg Weight	Shell Breaking Strength	Shell Thickness	Shell Weight	Shell Percentage	Deformation
2088	72109	62414	62288	2	7	1	54.80	3.58	332.70	5.12	9.34	54.21
2089	72110	62414	62288	2	7	1	56.12	3.76	348.30	5.35	9.54	55.73
2090	72113	62414	62470	2	7	1	63.02	3.70	365.30	6.15	9.76	51.57
2091	72114	62414	62470	2	7	1	58.97	3.91	369.30	5.88	9.98	48.89
2092	72115	62414	62470	2	7	1	55.35	3.80	358.00	5.56	10.05	47.57
2093	72118	62414	62470	2	7	1	55.70	3.91	372.70	5.71	10.25	45.38
2094	72119	62414	62470	2	7	2						
2095	72121	62414	62481	2	7	1	57.13	4.14	378.30	5.85	10.24	46.24
2096	72123	62414	62481	2	7	1	62.26	3.46	348.00	5.60	8.99	57.82
2097	72125	62414	62481	2	7	1	55.71	3.10	350.30	5.27	9.46	55.74
2098	72126	62414	62481	2	7	1	58.20	3.55	328.30	5.31	9.12	55.22
2099	72129	62414	62481	2	7	2						
2100	72130	62414	62500	2	7	1	56.18	3.76	347.00	5.51	9.80	52.47
2101	72132	62414	62500	2	7	1	52.58	3.55	353.30	5.24	9.96	52.71
2102	72134	62414	62558	2	7	1	55.66	4.21	371.00	5.63	10.11	43.73
2103	72136	62414	62558	2	7	1	59.92	3.56	341.30	5.51	9.20	55.19
2104	72137	62414	62558	2	7	1	51.56	4.13	338.70	5.03	9.75	51.76
2105	72140	62414	62584	2	7	1	56.83	3.53	345.70	5.47	9.63	54.61

Fig. 3.1. Screenshot of the spreadsheet containing individual records for the strong line, including pedigree information (from column A to C).

Figure 3.1 above is meant to give a general idea about how the records were organized before analysis. Naturally, this figure only shows a tiny part of the actual data; not only does the original spreadsheet contain thousands of lines, but many more columns containing the measurements of the remaining traits are not represented in this screenshot. Attentive readers may have noticed that the lines with no records (lines 2094 and 2099 in Figure 3.1) correspond to males (i.e. Sex = 2), and are therefore not missing records. In this regard, indicating information such as the sex of the individual facilitates the quick identification of potential errors and missing records.

The statistical analysis detailed in the following sections is based on the data put together in spreadsheets as explained above, with the aim to make its meaning more explicit than the many rows of numbers shown in Figure 3.1.

3.2) Means, standard deviations, and coefficients of variation

3.2.1) Generation means

In order to introduce the data simply, it is essential as a first step to provide a graphic perspective on the evolution of the phenotypic values over the course of the experiment –i.e. the 31 generations– for each of the eleven traits measured, for both lines. By displaying the whole data set, this first part aims to show some of the most direct effects of the selection process for non-destructive deformation and gives clues about its usefulness on a long-term perspective. At the same time, this part is also helpful to identify potential drawbacks from using a selection method based solely on a single eggshell trait.

To facilitate visualization and familiarization with the data, the generation means for all of the phenotypic traits throughout the 31 generations of the experiment, for both lines, are presented below (Table 3.1 and Table 3.2).

Table 3.1. Generation means for all of the phenotypic traits measured (strong line).

Gen [1]	n	NDD	BS	ST	SW	EW	SP	W	L	SI	EP	SM
1	412	N/A	2.98	326.4	5.1	58.6	8.7	4.19	5.91	71.1	87.0	143.8
2	284	59.9	2.95	319.4	5.0	58.0	8.7	4.22	5.78	73.1	83.9	138.9
3	275	54.9	2.97	320.4	5.0	57.3	8.8	4.25	5.75	74.0	83.7	137.8
4	266	61.2	3.13	329.9	5.0	56.4	8.9	4.18	5.70	73.5	81.9	141.6
5	301	55.1	3.44	344.4	5.4	58.3	9.3	4.22	5.74	73.6	79.0	143.4
6	297	54.4	3.52	335.9	5.3	57.1	9.2	4.18	5.70	73.3	80.0	149.0
7	290	54.2	3.60	344.7	5.2	55.5	9.5	4.13	5.67	72.8	80.2	143.2
8	288	49.9	3.65	354.1	5.4	55.6	9.7	4.14	5.65	73.4	75.7	157.3
9	292	53.4	3.61	339.0	5.1	54.2	9.4	4.11	5.59	73.5	77.0	153.6
10	288	49.6	3.68	352.9	5.4	55.4	9.8	4.12	5.69	72.4	71.8	154.6
11	248	51.9	3.76	357.3	5.3	53.1	10.0	4.05	5.60	72.3	73.0	151.6
12	254	47.1 [2]	3.84	359.3	5.4	53.9	10.1	4.08	5.61	72.8	71.4	158.7
13	198	43.6	4.00	363.3	5.4	53.3	10.2	4.07	5.58	73.1	73.4	153.4
14	130	42.0	3.95	364.8	5.5	53.5	10.4	4.09	5.58	73.2	75.7	157.3
15	136	41.4	3.95	365.9	5.4	52.3	10.3	4.05	5.54	73.3	68.4	159.6
16	146	41.4	4.00	363.3	5.3	51.4	10.3	4.03	5.47	73.8	72.7	157.1
17	150	41.7	4.25	369.2	5.4	51.5	10.5	4.06	5.46	74.4	70.5	162.6
18	135	37.7	4.43	378.7	5.5	50.8	10.8	4.07	5.40	75.5	N/A	N/A
19	146	38.8	4.43	378.2	5.6	52.3	10.8	4.16	5.47	76.0	N/A	N/A
20	118	40.0	4.34	372.5	5.6	52.6	10.6	4.15	5.51	75.5	N/A	N/A
21	123	39.8	4.53	370.8	5.4	50.3	10.8	4.10	5.38	76.1	N/A	N/A
22	114	40.1	4.48	379.2	5.5	51.3	10.8	4.10	5.44	75.4	N/A	N/A
23	250	38.9	4.61	377.7	5.5	51.2	10.7	4.10	5.43	75.5	N/A	N/A
24	218	43.9	4.45	371.1	5.5	52.5	10.5	4.14	5.49	75.4	N/A	N/A
25	160	43.9	4.42	367.1	5.5	52.6	10.4	4.08	5.58	73.1	N/A	N/A
26	228	44.3	4.40	371.9	5.7	54.2	10.5	4.11	5.65	72.7	N/A	N/A
27	215	42.7	4.42	369.3	5.6	53.1	10.5	4.08	5.60	72.9	N/A	N/A
28	137	44.0	4.61	370.3	5.5	52.7	10.5	4.07	5.57	73.1	N/A	N/A
29	161	39.7	4.85	381.4	5.6	51.6	10.9	4.04	5.49	73.7	N/A	N/A
30	146	39.6 [2]	4.72	380.0	5.6	51.7	10.8	4.03	5.55	72.6	N/A	N/A
31	113	40.6 [2]	4.61	374.0	5.6	52.3	10.8	4.09	5.51	74.1	N/A	N/A
Mean	210	45.9	4.02	359.7	5.4	53.7	10.1	4.11	5.58	73.7	76.8	150.8

[1] Gen, Generation; n, number of records; NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm); SW, Eggshell weight (g); EW, Egg weight (g); SP, Eggshell percentage (%); W, Egg width (cm); L, Egg length (cm); SI, Shape index (%); EP, Egg production (eggs/100 days); SM, Sexual maturity (days); N/A, Not available.

[2] The values for non-destructive deformation for generations 12, 30, and 31, were calculated using multiple-regression analysis, based on the other eggshell traits.

Table 3.2. Generation means for all of the phenotypic traits measured (weak line).

Gen [1]	n	NDD	BS	ST	SW	EW	SP	W	L	SI	EP	SM
1	412	N/A	2.98	326.4	5.1	58.6	8.7	4.19	5.91	71.1	87.0	143.8
2	300	64.7	2.77	307.8	4.8	57.0	8.4	4.19	5.77	72.7	84.0	137.5
3	268	60.7	2.75	306.7	4.7	55.5	8.5	4.21	5.72	73.6	85.5	139.2
4	258	68.7	2.81	313.7	4.6	54.1	8.4	4.11	5.64	73.0	85.3	139.3
5	280	69.0	2.83	312.2	4.8	56.4	8.5	4.17	5.71	73.0	84.1	139.9
6	291	72.5	2.68	294.9	4.5	55.0	8.1	4.12	5.69	72.5	89.1	145.4
7	271	76.5	2.62	295.3	4.3	53.7	8.1	4.09	5.63	72.6	90.9	135.4
8	265	75.1	2.50	293.1	4.4	53.9	8.1	4.11	5.60	73.4	88.4	144.0
9	283	81.9	2.46	279.9	4.1	52.7	7.7	4.07	5.56	73.3	89.9	138.7
10	277	90.1	2.22	264.2	3.8	51.8	7.4	4.04	5.60	72.1	85.0	135.2
11	250	100.6	2.17	257.7	3.7	50.5	7.3	4.01	5.53	72.6	88.0	132.5
12	250	97.6 [2]	2.03	251.7	3.6	50.5	7.1	4.02	5.53	72.7	85.6	131.1
13	187	101.0	1.98	248.2	3.5	50.3	7.0	4.01	5.52	72.7	88.1	138.7
14	136	98.5	1.97	248.2	3.6	52.3	6.9	4.07	5.61	72.5	92.0	142.0
15	129	105.4	1.83	240.6	3.4	51.0	6.6	4.03	5.58	72.4	85.5	135.5
16	140	107.6	1.81	238.4	3.4	50.4	6.7	4.00	5.58	71.9	89.3	135.2
17	97	98.5	2.15	249.3	3.5	50.4	7.0	4.04	5.52	73.2	87.1	140.4
18	91	88.9	2.32	262.1	3.7	50.6	7.3	4.08	5.47	74.6	N/A	N/A
19	132	95.5	2.14	251.1	3.7	51.7	7.1	4.17	5.51	75.8	N/A	N/A
20	82	95.4	2.16	248.5	3.6	51.2	7.1	4.15	5.50	75.6	N/A	N/A
21	121	95.1	2.22	253.8	3.6	49.1	7.3	4.10	5.40	75.9	N/A	N/A
22	66	97.7	2.08	250.5	3.5	48.6	7.1	4.05	5.40	75.1	N/A	N/A
23	175	94.8	2.18	255.1	3.5	49.7	7.1	4.07	5.49	74.2	N/A	N/A
24	185	105.5	2.14	250.2	3.5	49.9	7.1	4.09	5.48	74.8	N/A	N/A
25	104	100.3	2.19	255.3	3.6	50.8	7.2	4.05	5.56	72.9	N/A	N/A
26	193	103.2	2.23	254.4	3.7	52.0	7.1	4.07	5.63	72.4	N/A	N/A
27	124	100.1	2.24	252.3	3.6	49.9	7.2	4.01	5.54	72.4	N/A	N/A
28	135	97.9	2.28	258.3	3.8	52.2	7.2	4.06	5.64	72.1	N/A	N/A
29	167	90.5	2.40	264.6	3.8	50.6	7.5	4.04	5.52	73.2	N/A	N/A
30	160	93.3 [2]	2.36	259.7	3.7	50.6	7.3	4.01	5.60	71.6	N/A	N/A
31	74	92.6 [2]	2.41	259.4	3.7	50.4	7.4	4.03	5.56	72.5	N/A	N/A
Mean	190	90.6	2.32	267.9	3.9	52.0	7.5	4.08	5.58	73.2	87.3	138.5

[1] Gen, Generation; n, number of records; NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm); SW, Eggshell weight (g); EW, Egg weight (g); SP, Eggshell percentage (%); W, Egg width (cm); L, Egg length (cm); SI, Shape index (%); EP, Egg production (eggs/100 days); SM, Sexual maturity (days); N/A, Not available.

[2] The values for non-destructive deformation for generations 12, 30, and 31, were calculated using multiple-regression analysis, based on the other eggshell traits.

Non-destructive deformation

Given that it was the selection criterion used for the experiment, let us begin the analysis of phenotypic values with non-destructive deformation. From Figure 3.2a, which displays the entire data, the phenotypic values of the weak line exhibit a gradual increase, as compared to a decrease in the strong line. Even though this was expected given the selection process, which consisted in choosing the individuals with the highest deformation values as parents for the following generation in the weak line, and vice-versa for the strong line, it is already at this stage possible to conclude that the selection experiment itself was successful, given that two lines were created which are visibly distinct and evolved independently of each other.

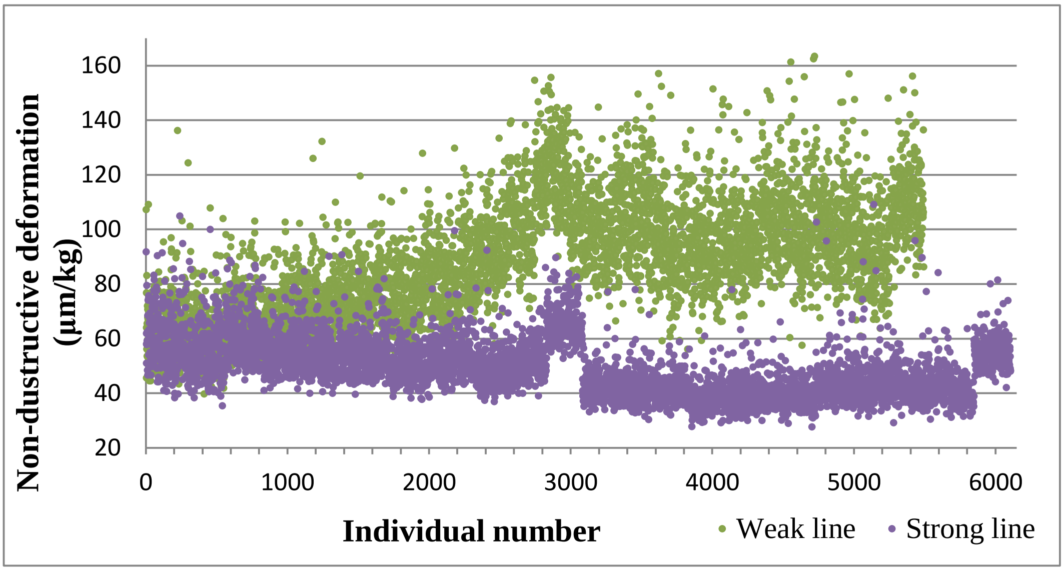

Fig. 3.2a. Evolution of the phenotypic values for non-destructive deformation over time.

From the original data (Figure 3.2a), it appears that the values of the individuals from generations 12, 30, and 31 –corresponding approximately to individuals 2750 to 3000 and 5250 to 5500 for the weak line, and individuals 2800 to 3050 and 5850 to 6100 for the strong line–, lie above the values which could be expected in their respective lines. These biased values arose because the measurements had to be made with a different machine for these generations due to technical difficulties. To obtain more realistic values, a multiple-regression analysis was performed based on the other eggshell traits. Though more detail will be given in the following section, these new values were used to calculate the generation means presented below (Figure 3.2b).

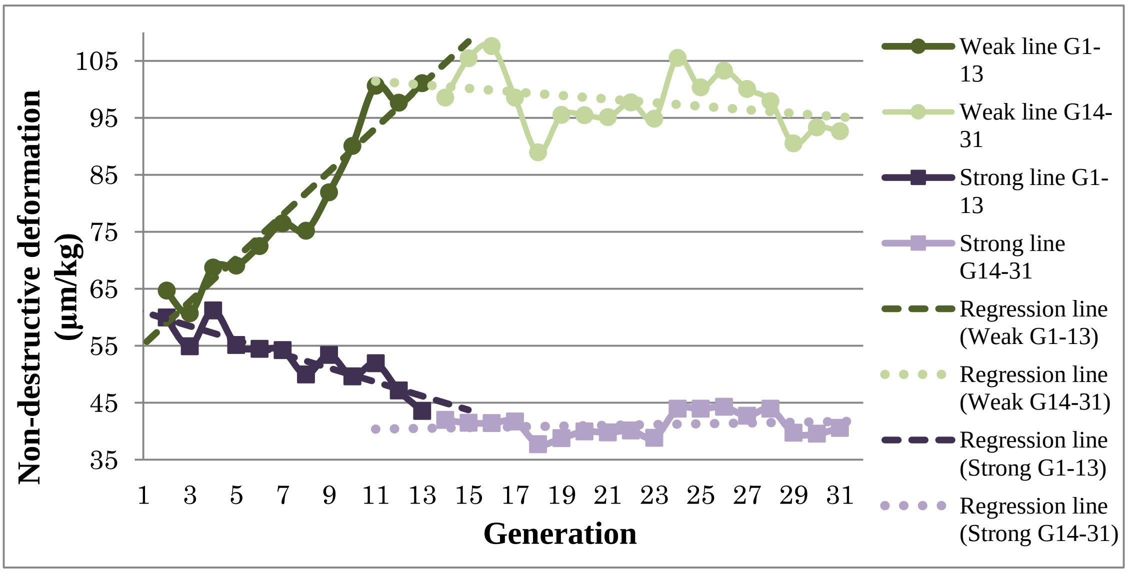

Fig. 3.2b. Evolution of the generation mean for non-destructive deformation over time.

The generation means represented by Figure 3.2b show more clearly the trend observed with Figure 3.2a. To facilitate understanding of the evolution of the phenotypic values, two regression lines were added to each line, one covering generations 1 to 13, period during which selection was intensive, and the other covering generations 14 to 31, after selection was relaxed and then stopped. The regression coefficients of the generation means were –1.22 μm/kg ($P < 0.001$) for the strong line and 3.80 μm/kg ($P < 0.0001$) for the weak line from generation 1 to generation 13, and 0.07 μm/kg ($P > 0.1$) for the strong line and –0.31 μm/kg ($P > 0.1$) for the weak line (Figure 3.2b) from generations 14 to 31. Concretely, these results imply that the response to intensive selection was asymmetric for non-destructive deformation, and that the phenotypic values stabilized after generation 14.

Eggshell breaking strength

One of the reasons for experimenting with non-destructive deformation in the first place is to evaluate whether or not it is a suitable indicator of eggshell strength, and in turn attempt to determine whether it could be used on a large scale instead of eggshell breaking strength, whose major drawback lies in the destruction of the eggs to assess their strength. In this regard, the effect of selection for non-destructive deformation on eggshell breaking strength is critical in this analysis, and it is necessary to closely examine the evolution of the phenotypic values of that trait over time.

As shown in Figure 3.3a, the selection process was effective and the two lines are unmistakably distinct from each other. Note that whereas the phenotypic values for non-destructive deformation declined over time in the strong line, as low deformation values indicate a strong eggshell, the trend is actually the opposite for eggshell breaking strength, which instead exhibits increasing values. This is simply because the stronger the eggshell, the higher the strength needed to break it. There is therefore a negative correlation between these two traits.

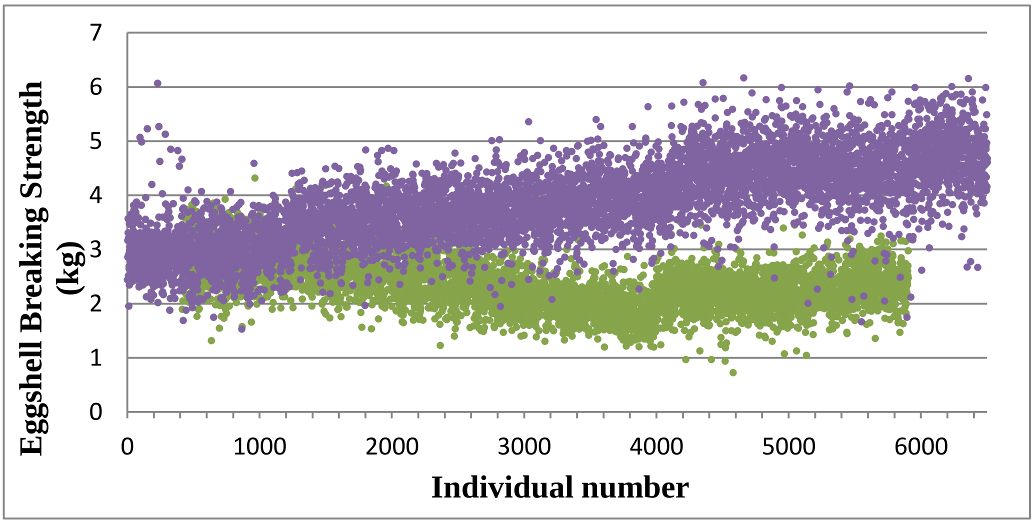

Fig. 3.3a. Evolution of the phenotypic values for eggshell breaking strength over time.

With respect to the generation means (Figure 3.3b), the trends detected are somewhat different to the observations made for non-destructive deformation. As a matter of fact, the regression coefficients for the period of intensive selection were 0.087 kg ($P < 0.0001$) for the strong line and –0.081 kg ($P < 0.0001$) for the weak line, and therefore response was symmetric, contrary to the observations made on non-destructive deformation. However, from generation 14 onward, while the generation means for non-destructive deformation had stabilized, i.e. displayed non-significant regression coefficients, with regard to eggshell breaking strength the mean of the strong line continued to increase (0.0388 kg, $P < 0.0001$) whereas that of the weak line started to move back toward the mean of the initial population (0.0253 kg, $P < 0.0001$). In other words, the response to selection was asymmetric in this case as well.

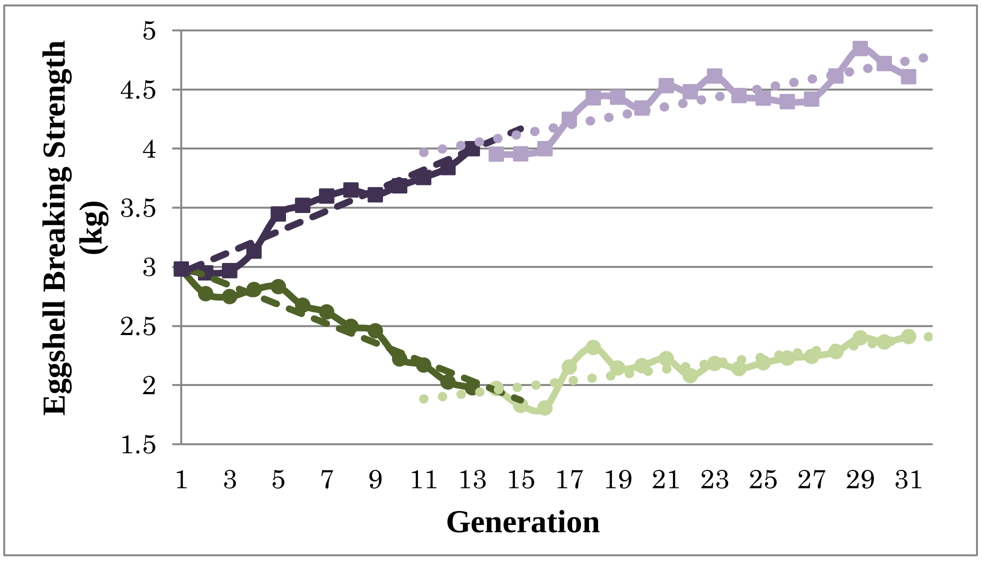

Fig. 3.3b. Evolution of the generation mean for eggshell breaking strength over time.

Other eggshell traits

Besides eggshell breaking strength, three other eggshell traits were measured to reflect the impact of selection on the strength of the eggshell: eggshell thickness, eggshell weight, and eggshell percentage. Similarly to eggshell breaking strength, the effect of selection is visible through the phenotypic values of the weak and strong lines, which diverged as the experiment progressed. More specifically, eggshell thickness, eggshell weight and eggshell percentage increased for the strong line, and decreased for the weak line

From generation 1 to 13, for eggshell thickness (Figure 3.4a and Figure 3.4b) and eggshell weight (Figure 3.5a and Figure 3.5b), the evolution of the data shows that the response to selection was similar to the movement displayed by non-destructive deformation, i.e. response was asymmetric. For eggshell thickness, the regression coefficient of the strong line was 3.54 μm ($P < 0.0001$), compared with –6.37 µm ($P < 0.0001$) for that of the weak line (Figure 3.4b). For eggshell weight, the regression coefficients were 0.033 g ($P < 0.01$) and –0.13 g ($P < 0.0001$) for the strong and weak lines, respectively (Figure 3.5b). Nevertheless, interestingly enough, from generation 14 onward (after selection was relaxed), the regression coefficients displayed the same tendency as did the phenotypic values of eggshell breaking strength, the strong line getting stronger, and the weak line moving back toward the mean of the initial population. Concretely, the regression coefficients were respectively 0.473 µm ($P < 0.06$) and 0.908 µm ($P < 0.001$) for the strong and weak lines for eggshell thickness, and 0.0114 g ($P < 0.01$) and 0.0142 g ($P < 0.01$) for eggshell weight.

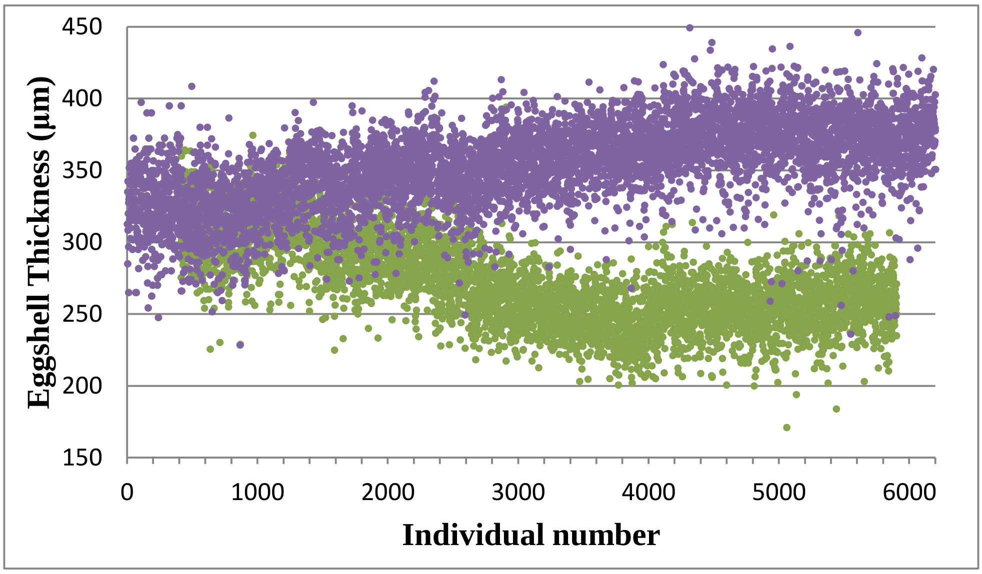

Fig. 3.4a. Evolution of the phenotypic values for eggshell thickness over time.

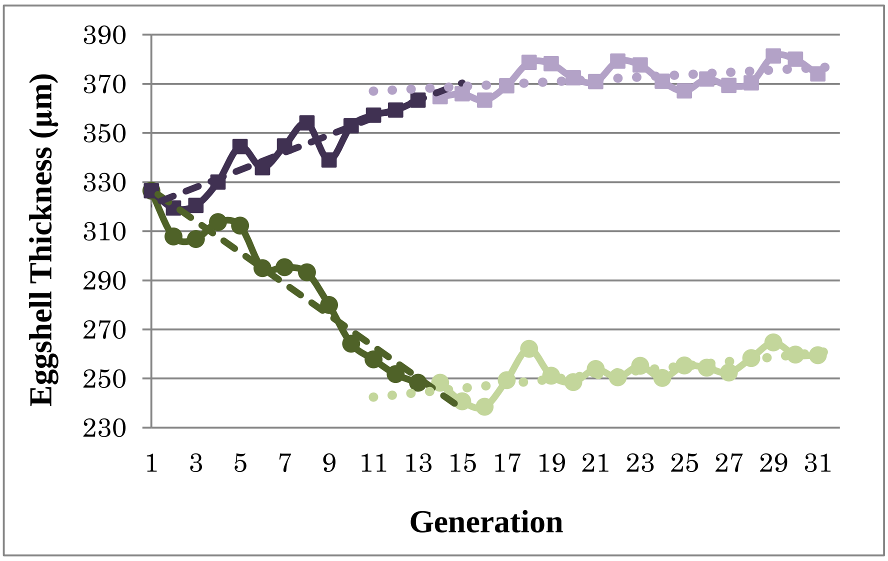

Fig. 3.4b. Evolution of the generation mean for eggshell thickness over time.

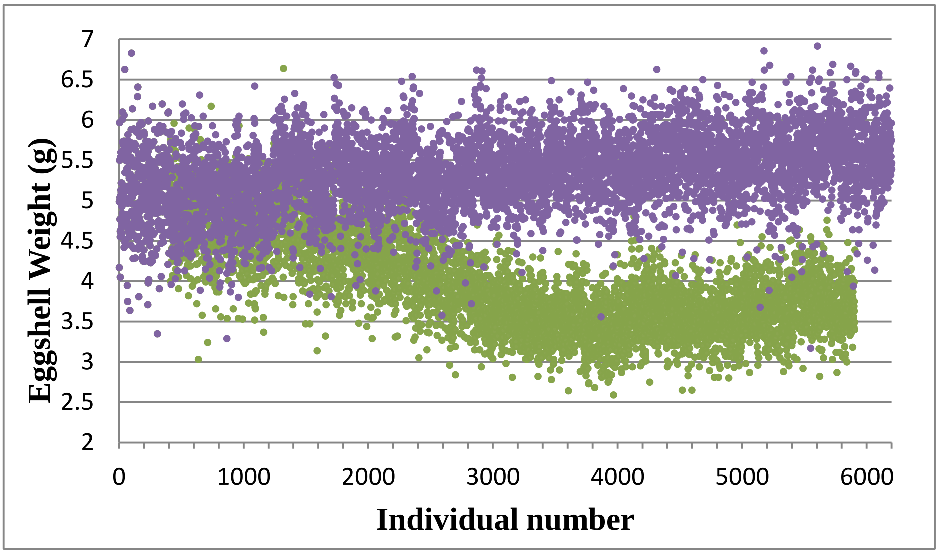

Fig. 3.5a. Evolution of the phenotypic values for eggshell weight over time.

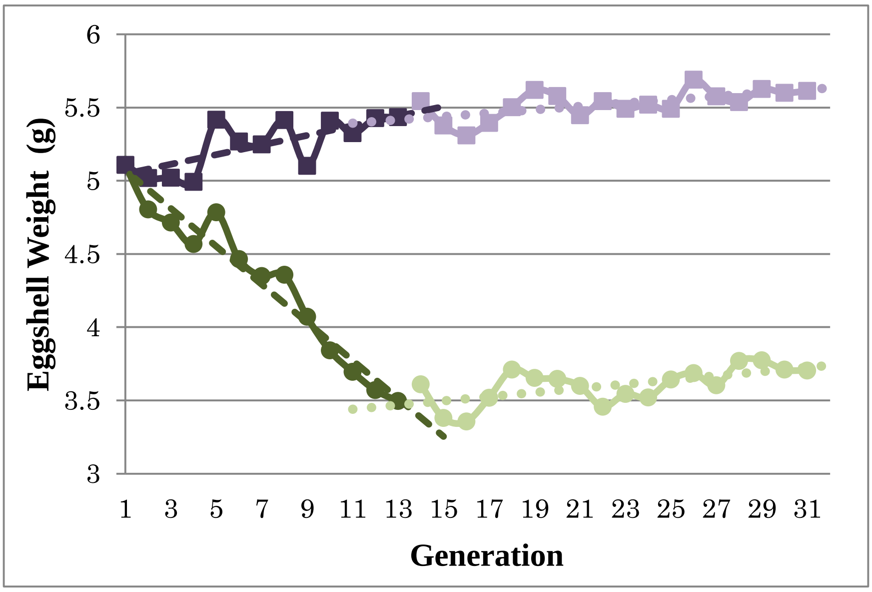

Fig. 3.5b. Evolution of the generation mean for eggshell weight over time.

For eggshell percentage (Figure 3.6a and Figure 3.6b), the phenotypic values displayed the same tendency already observed for eggshell breaking strength from generation 1 to 13. The regression coefficients for the period of intensive selection were similar for both lines: 0.135% ($P < 0.0001$) for the strong line and –0.147% ($P < 0.0001$) for the weak line. However, the response observed after relaxing and ceasing selection was asymmetric in this case as well, as the regression coefficients for generation 14 onward were 0.016% ($P < 0.07$) for the strong line and –0.031% ($P < 0.001$) for the weak line.

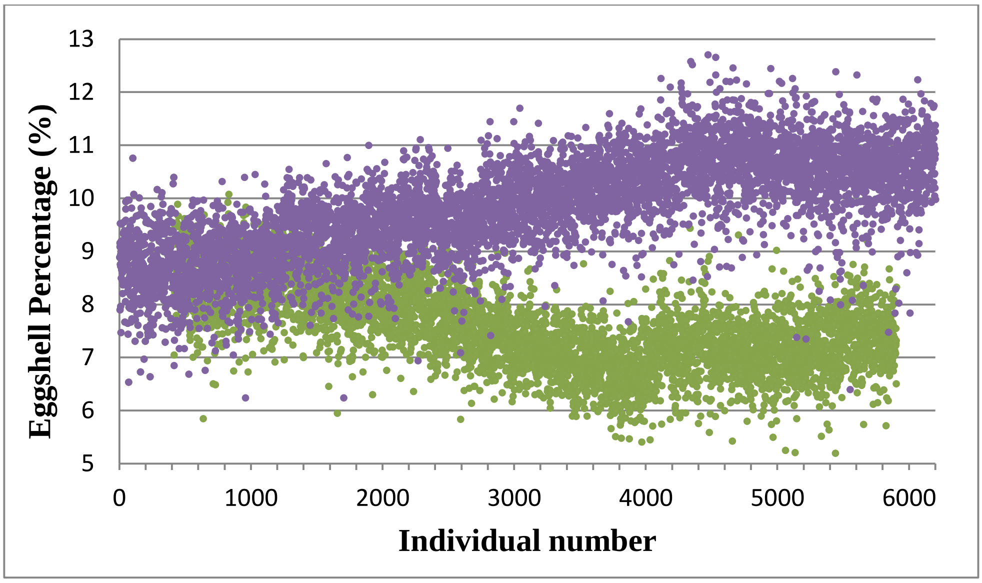

Fig. 3.6a. Evolution of the phenotypic values for eggshell percentage over time.

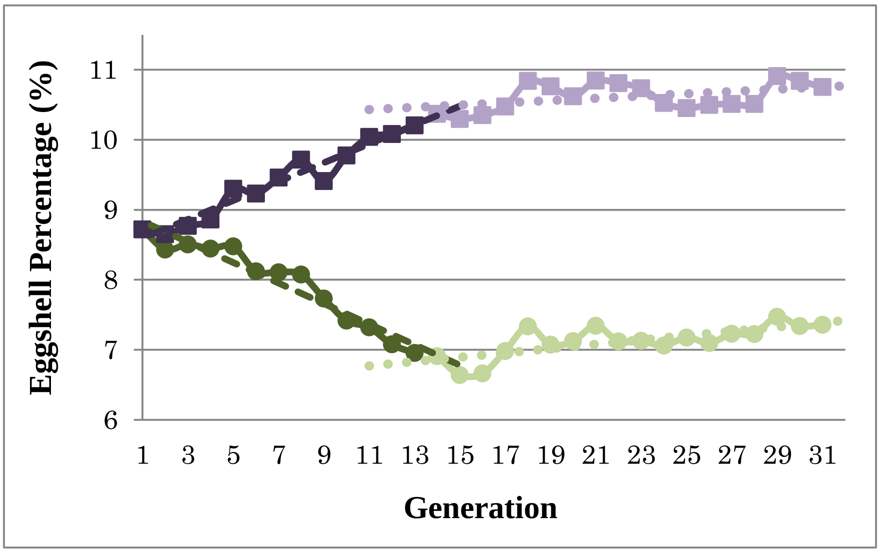

Fig. 3.6b. Evolution of the generation mean for eggshell percentage over time.

The analysis of the phenotypic values of the traits related to the quality of the eggshell performed above provides a few indications about the effects and effectiveness of using non-destructive deformation as a criterion for selection. First of all, the measurements made were precise enough to enable an effective selection process. Secondly, selection for non-destructive deformation proved to be an efficient tool for the creation of two lines according to the strength of their eggshell, thereby enabling the establishment of populations with stronger eggshells. In this regard, non-destructive deformation appeared to have the potential to replace other eggshell traits widely used for selection, such as eggshell breaking strength.

Nevertheless, as detailed above, the selection process did not have the same impact on all eggshell traits: not only did they respond differently to intensive selection, but the responses observed within each line after the end of the selection process also raise questions about the actual genetic improvement of the population of White Leghorn considered, which is one of the main goals of the experiment. To answer these questions, the coming chapters will provide a thorough analysis of the evolution of the breeding values and genetic parameters for these traits over the course of the experiment.

Egg weight

Plotting the data relative to egg weight revealed that selection for non-destructive deformation did not have the same effect on traits not directly related to the eggshell. As a matter of fact, as illustrated by Figure 3.7a, the measurements displayed a decline in egg weight for both lines, which is particularly conspicuous over the period of intensive selection. This may suggest that the selection process had little influence on egg weight, but there are other potential explanations for this phenomenon. Since egg-laying breeds like White Leghorns are generally selected for egg size or egg weight, it may be so that the base population used for this experiment, which stems from a larger population selected for commercial purposes, was intensively selected for egg weight. If the true genetic correlation between non-destructive deformation and egg weight is low, it is then possible that the selection process for non-destructive deformation allowed this population of White Leghorn to move back toward smaller phenotypic values for egg weight.

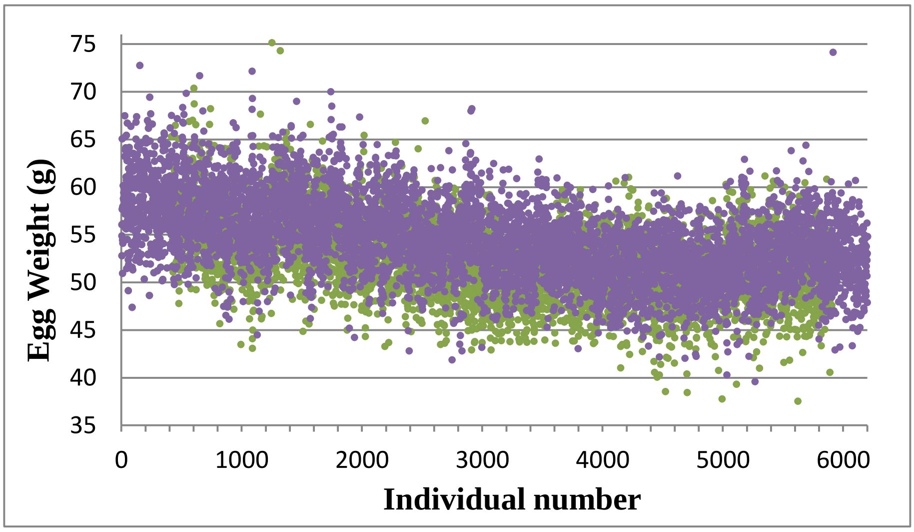

Fig. 3.7a. Evolution of the phenotypic values for egg weight over time.

The trend observed with the entire data appears more clearly by plotting the generation means (Figure 3.7b). From generation 1 to 13, the regression coefficient was –0.45 g ($P < 0.0001$) for the strong line, while it was –0.65 g per generation (P < 0.0001) for the weak line. However, after selection was relaxed, the regression coefficients changed to 0.03 g (P > 0.1) for the strong line and –0.02 g (P > 0.1) for the weak line. Analysis of the genetic parameters is necessary to determine the reason for this trend.

Furthermore, from Figure 3.7b, it appears that the difference in egg weight between the weak and strong lines is comprised between 1 and 3 g, the strong line displaying higher values. Given the analysis of eggshell weight above (Figure 3.5b), it is possible to infer that most of the difference in egg weight between the lines is due to the difference in eggshell weight.

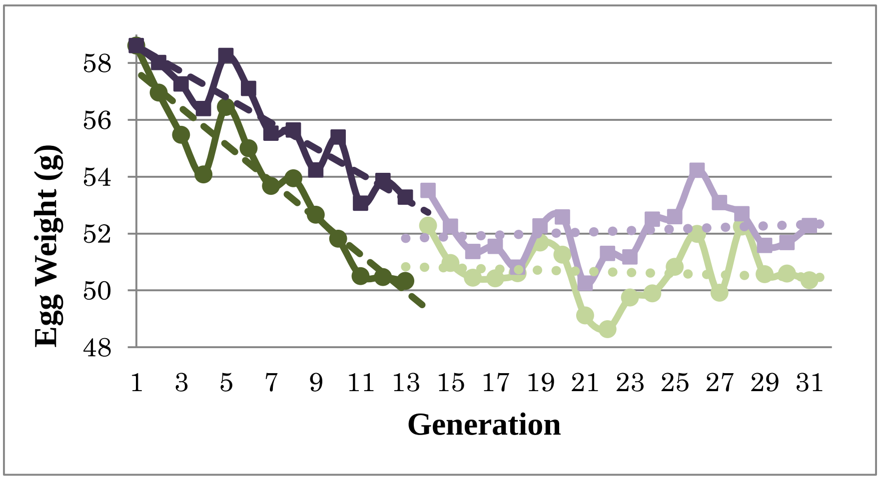

Fig. 3.7b. Evolution of the generation mean for egg weight over time.

Egg width, egg length, and egg shape index

The evolution of the generation means for egg width and egg length is presented below (Figure 3.8 and Figure 3.9).

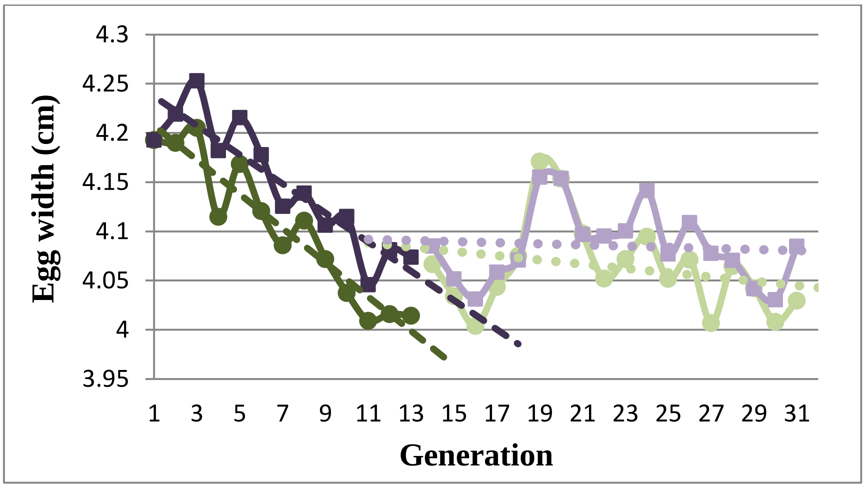

Fig. 3.8. Evolution of the generation mean for egg width over time.

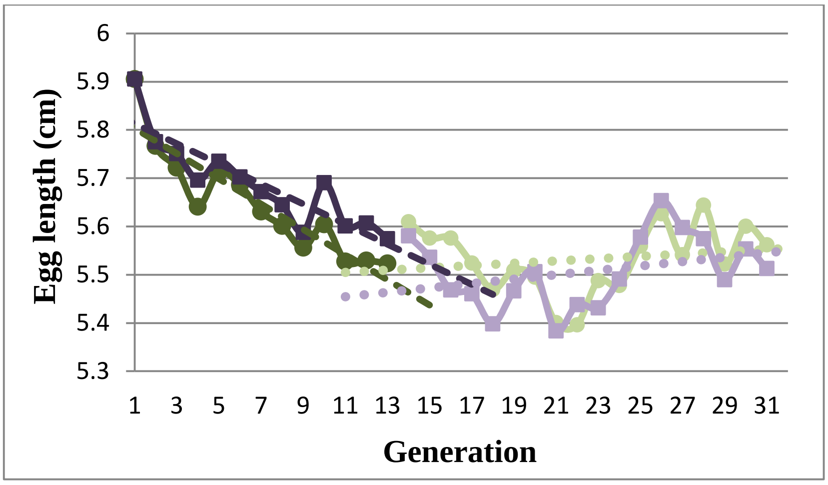

Fig. 3.9. Evolution of the generation mean for egg length over time.

These two graphs reveal a decrease in both egg width and egg length, in both lines. For generation 1 to generation 13, the regression coefficients for egg width were –0.0148 ($P < 0.0001$) for the strong line and –0.0174 ($P < 0.0001$) for the weak line. For egg length, these values were –0.0208 ($P < 0.0001$) for the strong line and –0.0262 ($P < 0.0001$) for the weak line. This trend, which indicates a decrease in egg size, is consistent with the decrease of the values observed for egg weight. Also, the evolution of the generation mean for the shape index revealed that both lines displayed similar trends (Figure 3.10). Further analysis is however needed to make relevant comments about this trend.

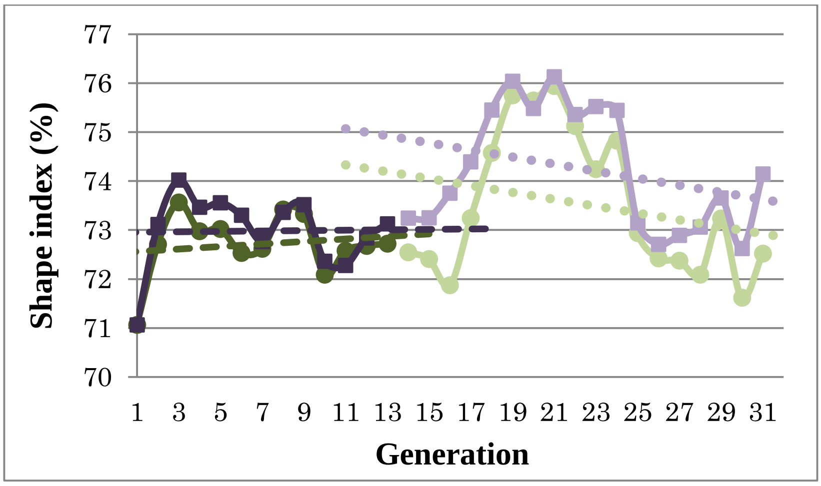

Fig. 3.10. Evolution of the generation mean for shape index over time.

Egg production

Unlike the other traits examined above, the data regarding egg production and sexual maturity was only available until generation 17, i.e. until the selection process ceased. For this reason, the total number of individuals is much lower for these two traits.

As illustrated by the plot representing the evolution of the number of eggs produced per individual per 100 days (Figure 3.11a), the strong line noticeably declined over time, implying that selection for strong eggshell had a negative impact on egg production traits. The trend is however much less apparent for the weak line.

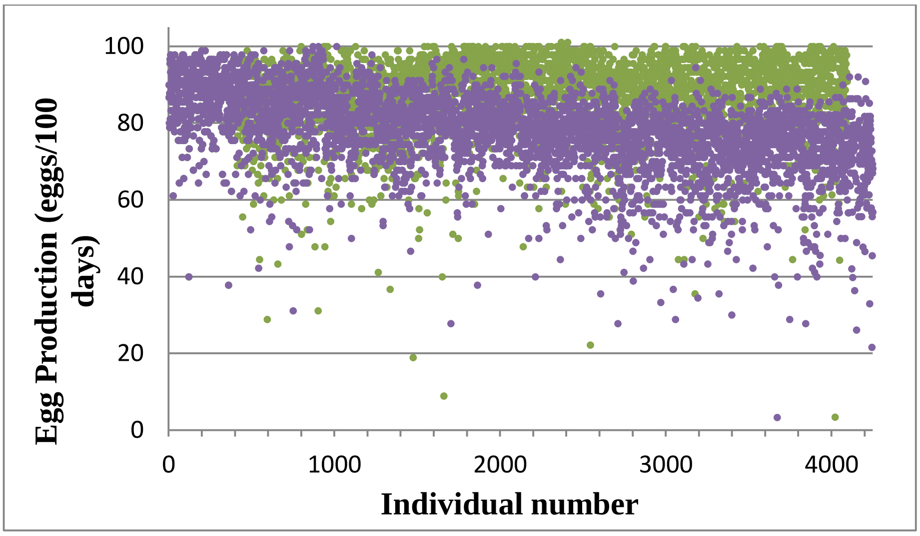

Fig. 3.11a. Evolution of the phenotypic values for egg production over time.

Figure 3.11b gives a better picture of the evolution of egg production over time. As aforementioned, the strong line displays a visible decline, with a regression coefficient of –1.24 eggs/100days (P < 0.0001) for generation 1 to 13. By contrast, the tendency for the weak line is much less pronounced, with a slight and non-significant increase of 0.19 eggs/100days per generation (P > 0.1). Although it is too early at this stage to draw any conclusions from these results, this may suggest that the population of White Leghorn considered is close to the selection limit, and may physiologically not be able to reach average levels of over 100 eggs per 100 days.

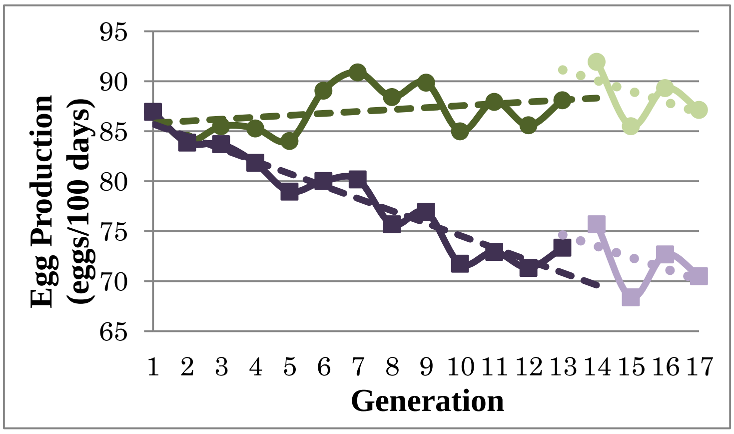

Fig. 3.11b. Evolution of the generation mean for egg production over time.

Sexual maturity

Sexual maturity is another factor of importance; it is a so-called production trait given the fact that chickens only start producing eggs after the onset of sexual maturity. Changes in sexual maturation can therefore have substantial repercussions on the number of eggs produced during the lifetime of an individual, and differences from one population to the other should not be overlooked. With this in mind, the current experiment revealed that selection for non-destructive deformation had an unmistakable impact on sexual maturity for both lines (Figure 3.12a and Figure 3.12b).

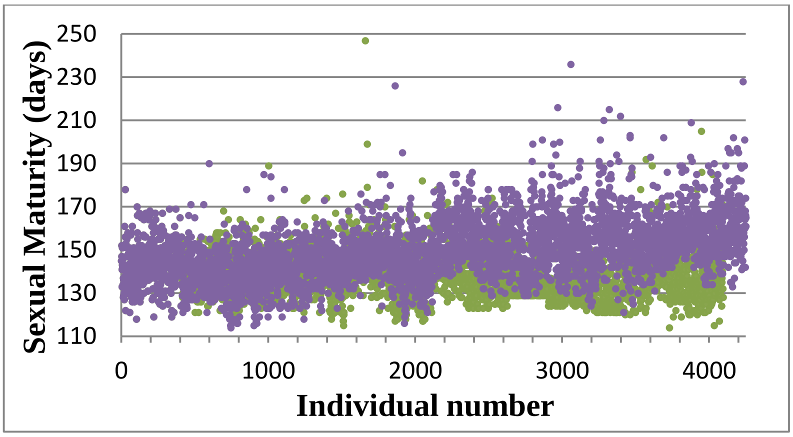

Fig. 3.12a. Evolution of the phenotypic values for sexual maturity over time.

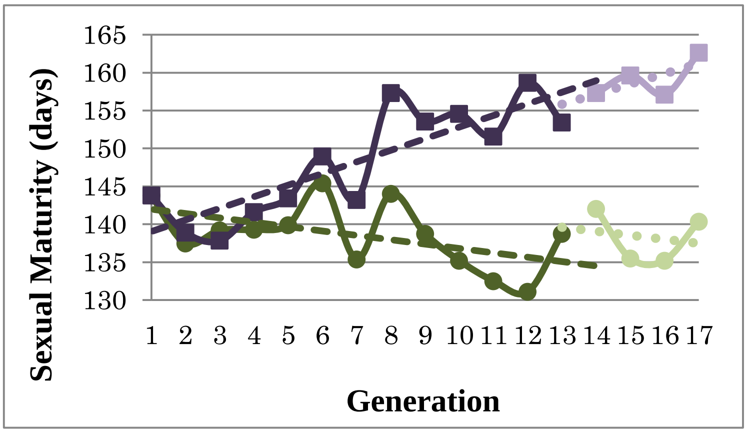

Fig. 3.12b. Evolution of the generation mean for sexual maturity over time.

As illustrated by Figure 3.12b above, the data displays an asymmetric response in this case as well, the regression coefficients being 1.53 days ($P < 0.001$) for the strong line and –0.58 days ($P < 0.05$) for the weak line for generations 1 to 13.

Through this simple analysis, it is already possible to assert that non-destructive deformation is a useful criterion for the assessment of eggshell strength, but at the same time some of the “negative” (from an industrial perspective) impacts of selection for stronger eggshell were also visible, in particular with regard to egg production traits. A more detailed analysis is however necessary, not only to better understand the reasons behind the evolution of the phenotypic values presented in this section, but also to quantify the extent of the effects of selection for non-destructive deformation on the various egg traits. These aspects will be examined in the following chapters.

3.2.2) Standard deviations and coefficients of variation

The standard deviations for both lines are summarized in Table 3.3 and Table 3.4.

Table 3.3. Standard deviations per generation for all of the phenotypic traits measured (strong line).

Gen [1]	n	NDD	BS	ST	SW	EW	SP	W	L	SI	EP	SM
1	412	N/A	0.51	23.8	0.50	3.9	0.64	0.11	0.18	2.3	8.0	9.8
2	284	10.4	0.45	23.3	0.45	3.9	0.59	0.11	0.20	2.5	8.7	9.2
3	275	9.5	0.43	21.8	0.44	3.8	0.59	0.10	0.20	2.5	9.9	10.6
4	266	8.3	0.38	17.4	0.37	3.6	0.52	0.10	0.17	2.2	7.3	10.3
5	301	7.3	0.43	20.3	0.40	3.3	0.56	0.09	0.16	2.3	7.9	8.0
6	297	7.0	0.44	20.3	0.43	3.9	0.56	0.11	0.17	2.1	7.7	8.8
7	290	7.2	0.43	20.3	0.40	3.4	0.56	0.10	0.16	2.1	6.7	11.7
8	288	5.6	0.40	20.1	0.41	3.7	0.55	0.10	0.18	2.0	7.5	10.8
9	292	7.1	0.43	19.8	0.36	2.9	0.54	0.08	0.15	1.9	8.5	10.5
10	288	6.7	0.45	20.6	0.44	3.8	0.56	0.12	0.19	2.5	10.2	15.2
11	248	6.2	0.46	19.1	0.37	3.2	0.55	0.10	0.17	2.2	9.5	13.0
12	254	5.1 [2]	0.43	18.8	0.35	3.0	0.50	0.09	0.16	2.1	9.2	15.2
13	198	5.2	0.45	17.6	0.33	2.9	0.48	0.09	0.15	2.2	10.0	10.3
14	130	4.3	0.38	17.4	0.37	3.1	0.51	0.09	0.16	1.9	7.7	9.2
15	136	6.3	0.49	22.2	0.38	2.6	0.62	0.09	0.15	2.4	11.4	10.8
16	146	4.7	0.42	16.7	0.33	3.0	0.50	0.09	0.14	2.0	7.8	12.4
17	150	5.0	0.47	20.5	0.38	2.9	0.59	0.09	0.16	2.3	11.8	14.7
18	135	4.6	0.55	20.6	0.39	2.9	0.62	0.09	0.15	2.2	N/A	N/A
19	146	4.7	0.53	20.5	0.38	3.0	0.63	0.10	0.14	2.0	N/A	N/A
20	118	6.7	0.49	22.4	0.41	3.0	0.69	0.09	0.16	2.1	N/A	N/A
21	123	4.9	0.51	20.4	0.39	3.0	0.60	0.10	0.14	2.2	N/A	N/A
22	114	5.0	0.53	20.0	0.38	2.8	0.53	0.09	0.16	2.2	N/A	N/A
23	250	4.1	0.47	22.7	0.36	3.0	0.55	0.10	0.14	2.0	N/A	N/A
24	218	7.6	0.55	22.2	0.44	3.5	0.68	0.11	0.15	2.1	N/A	N/A
25	160	8.1	0.58	25.9	0.44	3.1	0.71	0.10	0.18	2.6	N/A	N/A
26	228	7.6	0.57	22.6	0.41	3.1	0.65	0.09	0.16	2.2	N/A	N/A
27	215	7.3	0.60	23.1	0.44	3.5	0.64	0.11	0.18	2.4	N/A	N/A
28	137	6.5	0.56	22.3	0.43	3.2	0.66	0.10	0.16	2.3	N/A	N/A
29	161	4.3	0.51	17.2	0.39	3.1	0.47	0.09	0.15	2.0	N/A	N/A
30	146	4.8 [2]	0.59	20.1	0.40	3.1	0.53	0.10	0.15	2.2	N/A	N/A
31	113	5.1 [2]	0.48	17.7	0.33	2.9	0.66	0.08	0.15	2.1	N/A	N/A
Mean	210	6.2	0.48	20.6	0.40	3.2	0.58	0.10	0.16	2.2	8.8	11.2

[1] Gen, Generation; n, number of records; NDD, Non-destructive deformation (µm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (µm); SW, Eggshell weight (g); EW, Egg weight (g); SP, Eggshell percentage (%); W, Egg width (cm); L, Egg length (cm); SI, Shape index (%); EP, Egg production (eggs/100 days); SM, Sexual maturity (days); N/A, Not available.

[2] The values for non-destructive deformation for generations 12, 30, and 31, were calculated using multiple-regression analysis, based on the other eggshell traits.

Table 3.4. Standard deviations per generation for all of the phenotypic traits measured (weak line).

Gen[1]	n	NDD	BS	ST	SW	EW	SP	W	L	SI	EP	SM
1	412	N/A	0.51	23.8	0.50	3.9	0.64	0.11	0.18	2.3	8.0	9.8
2	300	11.8	0.45	22.8	0.45	3.7	0.58	0.10	0.18	2.4	9.2	8.1
3	268	10.9	0.42	21.1	0.40	3.7	0.61	0.11	0.19	2.7	10.2	9.2
4	258	9.9	0.34	19.5	0.40	3.5	0.51	0.11	0.17	2.3	8.5	9.0
5	280	10.0	0.40	21.5	0.44	3.8	0.55	0.11	0.17	2.4	10.3	10.9
6	291	11.2	0.37	20.9	0.41	3.4	0.57	0.10	0.19	2.6	10.0	10.9
7	271	11.1	0.42	19.7	0.38	3.4	0.57	0.11	0.17	2.5	7.4	9.9
8	265	10.6	0.39	20.1	0.39	3.3	0.52	0.11	0.14	2.1	8.9	8.6
9	283	11.9	0.41	20.2	0.36	3.3	0.52	0.12	0.16	2.6	9.5	9.7
10	277	12.0	0.33	19.3	0.31	3.2	0.47	0.11	0.16	2.4	8.9	6.2
11	250	13.4	0.34	17.1	0.32	3.3	0.48	0.11	0.16	2.3	8.6	7.0
12	250	8.6[2]	0.30	14.9	0.29	3.0	0.42	0.10	0.15	2.1	10.7	7.6
13	187	12.2	0.26	14.9	0.26	2.7	0.47	0.09	0.14	2.1	8.1	10.5
14	136	12.3	0.27	15.4	0.30	3.1	0.44	0.11	0.15	2.4	6.6	8.6
15	129	13.9	0.30	15.8	0.30	3.5	0.46	0.11	0.18	2.6	9.2	9.5
16	140	16.4	0.29	17.5	0.30	3.1	0.52	0.10	0.17	2.6	8.3	11.9
17	97	15.2	0.33	17.3	0.30	2.8	0.49	0.09	0.16	2.2	12.5	11.3
18	91	14.0	0.35	18.4	0.37	3.3	0.54	0.11	0.18	3.0	N/A	N/A
19	132	14.0	0.39	17.4	0.32	3.3	0.49	0.10	0.17	2.5	N/A	N/A
20	82	14.1	0.37	17.7	0.30	3.1	0.57	0.11	0.18	2.7	N/A	N/A
21	121	15.9	0.37	17.4	0.29	3.4	0.57	0.11	0.20	3.0	N/A	N/A
22	66	13.6	0.33	14.9	0.24	3.4	0.45	0.16	0.17	3.2	N/A	N/A
23	175	12.9	0.30	15.7	0.29	3.1	0.47	0.10	0.16	2.4	N/A	N/A
24	185	13.8	0.31	16.8	0.29	3.1	0.46	0.10	0.19	2.9	N/A	N/A
25	104	17.8	0.36	20.6	0.35	3.4	0.62	0.11	0.19	2.6	N/A	N/A
26	193	15.6	0.34	19.0	0.31	3.5	0.51	0.11	0.18	2.7	N/A	N/A
27	124	15.0	0.34	17.3	0.28	3.4	0.52	0.11	0.20	2.9	N/A	N/A
28	135	15.6	0.33	19.8	0.33	3.2	0.57	0.10	0.19	2.8	N/A	N/A
29	167	13.4	0.33	17.8	0.31	3.5	0.53	0.11	0.16	2.2	N/A	N/A
30	160	12.4[2]	0.33	16.6	0.31	3.2	0.50	0.10	0.18	2.6	N/A	N/A
31	74	12.0[2]	0.32	17.3	0.29	2.8	0.46	0.10	0.17	2.8	N/A	N/A
Mean	190	13.1	0.35	18.3	0.33	3.3	0.52	0.11	0.17	2.5	9.1	9.3

[1] Gen, Generation; n, number of records; NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm); SW, Eggshell weight (g); EW, Egg weight (g); SP, Eggshell percentage (%); W, Egg width (cm); L, Egg length (cm); SI, Shape index (%); EP, Egg production (eggs/100 days); SM, Sexual maturity (days); N/A, Not available.

[2] The values for non-destructive deformation for generations 12, 30, and 31, were calculated using multiple-regression analysis, based on the other eggshell traits.

The values presented in these tables are a valuable indication of the variability of the data for a given trait and generation. However, in this case, it is also important to keep in mind that using the standard deviations only is not sufficient to enable a proper comparison between the different generations for a specific phenotypic trait. Given that the generation means significantly changed over the course of the experiment for all of the phenotypic traits considered, it is usually considered that the coefficient of variation, calculated as the ratio of the standard deviation to the mean, should be used instead, as it takes into account the value of the mean and therefore provides a better estimate of the variability of the population over time.

For instance, although Table 3.3 indicates that the standard deviation for non-destructive deformation substantially decreased between generation 1 and generation 13 in the strong line, it is not possible to infer whether this can be attributed to a true decrease in variability, given that the generation mean corresponding to these values also decreased dramatically over the same period of time.

For this reason, the coefficients of variation were also calculated for both lines (Table 3.5 and Table 3.6). Although much could be said about these tables, the main comments to be made include the fact that the coefficients of variation decreased for eggshell traits in the strong line during the period of intensive selection, whereas the values for egg production increased.

Table 3.5. Coefficients of variation per generation for all of the phenotypic traits measured (strong line).

Gen [1]	n	NDD	BS	ST	SW	EW	SP	W	L	SI	EP	SM
1	412	N/A	17.09	7.29	9.72	6.63	7.35	2.64	2.98	3.27	9.22	6.79
2	284	17.44	15.14	7.29	8.90	6.79	6.77	2.54	3.48	3.36	10.40	6.63
3	275	17.29	14.64	6.79	8.82	6.55	6.77	2.31	3.44	3.38	11.87	7.71
4	266	13.64	12.17	5.28	7.44	6.41	5.83	2.46	2.92	2.95	8.87	7.25
5	301	13.24	12.41	5.89	7.46	5.68	6.03	2.25	2.87	3.06	10.03	5.56
6	297	12.88	12.45	6.04	8.11	6.79	6.05	2.67	2.96	2.85	9.61	5.90
7	290	13.31	11.97	5.90	7.54	6.10	5.88	2.42	2.78	2.85	8.30	8.17
8	288	11.19	11.03	5.67	7.49	6.66	5.69	2.49	3.10	2.66	9.88	6.84
9	292	13.25	12.00	5.85	7.06	5.30	5.73	2.04	2.63	2.63	11.10	6.83
10	288	13.49	12.31	5.84	8.10	6.94	5.68	2.89	3.27	3.39	14.25	9.84
11	248	12.01	12.38	5.34	6.92	6.07	5.52	2.36	3.00	3.02	13.09	8.56
12	254	10.83 [2]	11.10	5.22	6.50	5.55	4.99	2.22	2.81	2.90	12.91	9.57
13	198	11.98	11.22	4.85	6.16	5.36	4.66	2.23	2.67	3.00	13.61	6.74
14	130	10.22	9.64	4.77	6.62	5.72	4.90	2.09	2.85	2.60	10.11	5.84
15	136	15.23	12.45	6.08	7.02	4.93	5.98	2.10	2.70	3.28	16.66	6.79
16	146	11.43	10.55	4.60	6.29	5.80	4.81	2.30	2.56	2.69	10.73	7.88
17	150	11.97	11.00	5.56	7.09	5.68	5.66	2.25	2.90	3.04	16.70	9.07
18	135	12.14	12.33	5.44	7.03	5.67	5.72	2.25	2.77	2.92	N/A	N/A
19	146	12.12	11.85	5.41	6.79	5.78	5.81	2.38	2.51	2.57	N/A	N/A
20	118	16.65	11.22	6.00	7.28	5.67	6.53	2.17	2.92	2.80	N/A	N/A
21	123	12.34	11.17	5.51	7.08	5.98	5.55	2.56	2.58	2.94	N/A	N/A
22	114	12.40	11.92	5.27	6.86	5.42	4.91	2.12	2.94	2.96	N/A	N/A
23	250	10.64	10.28	6.02	6.58	5.89	5.09	2.42	2.61	2.59	N/A	N/A
24	218	17.33	12.39	5.99	8.05	6.67	6.49	2.70	2.74	2.84	N/A	N/A
25	160	18.36	13.08	7.07	7.96	5.98	6.76	2.44	3.21	3.53	N/A	N/A
26	228	17.09	12.88	6.09	7.27	5.63	6.16	2.20	2.86	2.96	N/A	N/A
27	215	17.14	13.56	6.25	7.80	6.65	6.06	2.61	3.13	3.36	N/A	N/A
28	137	14.84	12.11	6.03	7.70	6.06	6.23	2.41	2.89	3.15	N/A	N/A
29	161	10.95	10.55	4.52	6.87	5.92	4.29	2.11	2.77	2.66	N/A	N/A
30	146	12.12 [2]	12.46	5.29	7.07	5.98	4.88	2.36	2.78	3.10	N/A	N/A
31	113	12.56 [2]	10.38	4.73	5.87	5.55	6.11	1.90	2.71	2.80	N/A	N/A
Mean	210	13.54	12.12	5.74	7.34	5.99	5.77	2.35	2.88	2.97	11.61	7.41

[1] Gen, Generation; n, number of records; NDD, Non-destructive deformation (µm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (µm); SW, Eggshell weight (g); EW, Egg weight (g); SP, Eggshell percentage (%); W, Egg width (cm); L, Egg length (cm); SI, Shape index (%); EP, Egg production (eggs/100 days); SM, Sexual maturity (days); N/A, Not available.

[2] The values for non-destructive deformation for generations 12, 30, and 31, were calculated using multiple-regression analysis, based on the other eggshell traits.

Table 3.6. Coefficients of variation per generation for all of the phenotypic traits measured (weak line).

Gen [1]	n	NDD	BS	ST	SW	EW	SP	W	L	SI	EP	SM
1	412	N/A	17.09	7.29	9.72	6.63	7.35	2.64	2.98	3.27	9.22	6.79
2	300	18.22	16.22	7.42	9.38	6.50	6.82	2.42	3.18	3.29	10.94	5.90
3	268	17.90	15.15	6.87	8.59	6.62	7.21	2.68	3.35	3.70	11.93	6.62
4	258	14.45	12.08	6.23	8.81	6.39	6.00	2.65	2.95	3.21	9.97	6.50
5	280	14.47	14.28	6.88	9.16	6.74	6.44	2.56	3.05	3.27	12.30	7.79
6	291	15.49	13.76	7.08	9.18	6.22	6.97	2.53	3.29	3.60	11.25	7.50
7	271	14.58	15.85	6.67	8.75	6.28	7.01	2.73	2.99	3.50	8.19	7.33
8	265	14.15	15.58	6.85	8.94	6.17	6.38	2.64	2.53	2.91	10.11	5.99
9	283	14.52	16.80	7.20	8.84	6.32	6.71	2.99	2.83	3.61	10.56	7.00
10	277	13.33	14.70	7.32	8.02	6.15	6.39	2.67	2.87	3.40	10.43	4.59
11	250	13.31	15.88	6.62	8.53	6.56	6.54	2.65	2.91	3.19	9.72	5.28
12	250	8.81 [2]	14.58	5.92	8.09	5.94	5.89	2.39	2.68	2.95	12.45	5.81
13	187	12.09	13.12	6.00	7.38	5.46	6.76	2.30	2.59	2.87	9.17	7.58
14	136	12.50	13.93	6.21	8.37	6.01	6.33	2.65	2.60	3.28	7.22	6.03
15	129	13.18	16.20	6.57	8.74	6.78	6.95	2.80	3.16	3.53	10.72	7.01
16	140	15.29	16.09	7.33	9.07	6.09	7.77	2.50	3.04	3.58	9.32	8.80
17	97	15.42	15.47	6.93	8.41	5.60	7.03	2.14	2.85	2.99	14.35	8.06
18	91	15.72	15.20	7.02	9.92	6.51	7.43	2.82	3.22	4.00	N/A	N/A
19	132	14.62	18.04	6.92	8.62	6.42	6.91	2.44	3.08	3.24	N/A	N/A
20	82	14.78	17.15	7.11	8.21	6.02	7.96	2.61	3.23	3.52	N/A	N/A
21	121	16.73	16.79	6.84	8.00	6.88	7.78	2.73	3.77	3.95	N/A	N/A
22	66	13.91	15.76	5.95	7.02	7.01	6.32	3.88	3.11	4.21	N/A	N/A
23	175	13.56	13.82	6.14	8.20	6.22	6.60	2.41	2.91	3.17	N/A	N/A
24	185	13.13	14.68	6.71	8.21	6.24	6.56	2.43	3.48	3.82	N/A	N/A
25	104	17.74	16.61	8.08	9.58	6.67	8.60	2.68	3.42	3.60	N/A	N/A
26	193	15.07	15.16	7.45	8.31	6.83	7.23	2.82	3.21	3.78	N/A	N/A
27	124	15.03	15.36	6.86	7.85	6.72	7.21	2.79	3.57	4.07	N/A	N/A
28	135	15.89	14.66	7.66	8.73	6.21	7.89	2.51	3.32	3.85	N/A	N/A
29	167	14.83	13.62	6.73	8.16	6.99	7.12	2.76	2.98	3.07	N/A	N/A
30	160	13.29 [2]	13.98	6.40	8.42	6.33	6.79	2.52	3.16	3.58	N/A	N/A
31	74	12.96 [2]	13.39	6.68	7.69	5.57	6.18	2.40	3.04	3.92	N/A	N/A
Mean	190	14.50	15.19	6.84	8.55	6.36	6.94	2.64	3.07	3.48	10.46	6.74

[1] Gen, Generation; n, number of records; NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm); SW, Eggshell weight (g); EW, Egg weight (g); SP, Eggshell percentage (%); W, Egg width (cm); L, Egg length (cm); SI, Shape index (%); EP, Egg production (eggs/100 days); SM, Sexual maturity (days); N/A, Not available.

[2] The values for non-destructive deformation for generations 12, 30, and 31, were calculated using multiple-regression analysis, based on the other eggshell traits.

3.3) Multiple-regression analysis

As mentioned in the tables describing the generation mean for non-destructive deformation (Table 3.1 and Table 3.2), the phenotypic values for non-destructive deformation for generations 12, 30, and 31 were calculated using multiple-regression analysis. As a matter of fact, because of technical problems, a different device had to be used to make the measurements for these generations, leading in turn to biased values (Figure 3.13a).

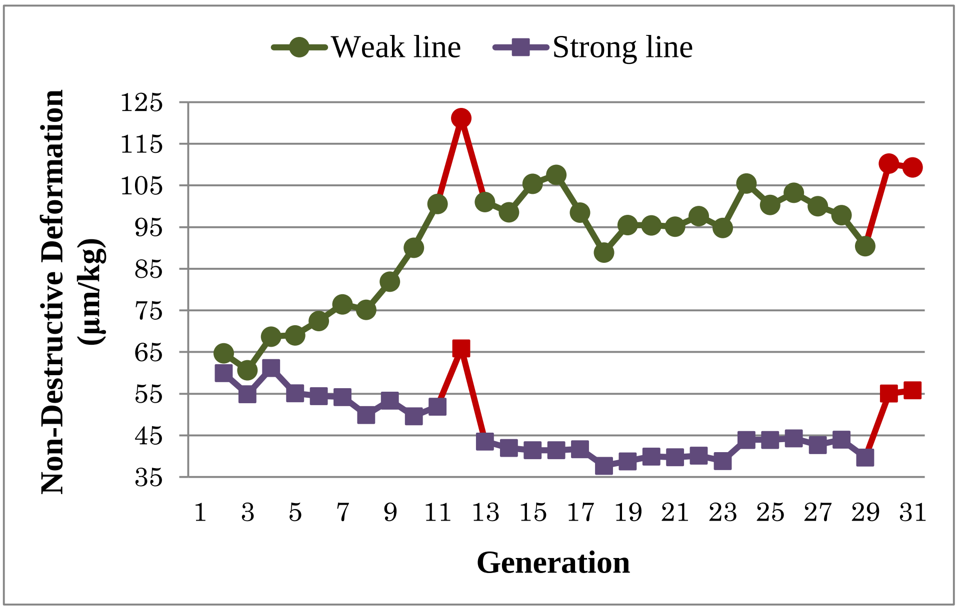

Fig. 3.13a. Generation mean for non-destructive deformation with the initial values.

Although this bias has no repercussion on the analysis of genetic parameters –i.e. the main analysis of this thesis– considering that it is absorbed as a fixed effect in a mixed animal model, multiple-regression methods are useful to calculate reasonably reliable estimates for these values. Three traits that are in general thought to have a high correlation with non-destructive deformation were chosen for the model: eggshell breaking strength, eggshell thickness, and eggshell percentage. In mathematical terms, the following regression equation was used

$$\hat{y} = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \qquad (3.1)$$

where

$\hat{y}$ is the estimate for non-destructive deformation,

α represents the intercept,

x_i represents the i^{th} independent variable (the i^{th} trait), and

β_i represents the regression coefficients corresponding to x_i.

Using this regression model, two equations were calculated for each line. The data from generation 1 to generation 11 was used to estimate generation 12, while generations 30 and 31 were estimated based on the data collected for generations 18 to 29. Because the intensity of selection and the properties of the eggshell changed as the experiment progressed, it was deemed necessary to calculate two equations per line in order to account for the slight changes in the relationship between the traits that may have occurred over the course of the experiment. As mentioned at the beginning of Chapter II, although the individuals were intensively selected during the first thirteen generations, a within-family selection procedure was used from generation 14 to 17, and from generation 18 onward, individuals were randomly chosen and mated within their lines.

The new generation means, which were calculated based on the estimated phenotypic value of each individual for these generations, are presented below (Figure 3.12b). Note that these values are identical to those presented in Figure 3.2b.

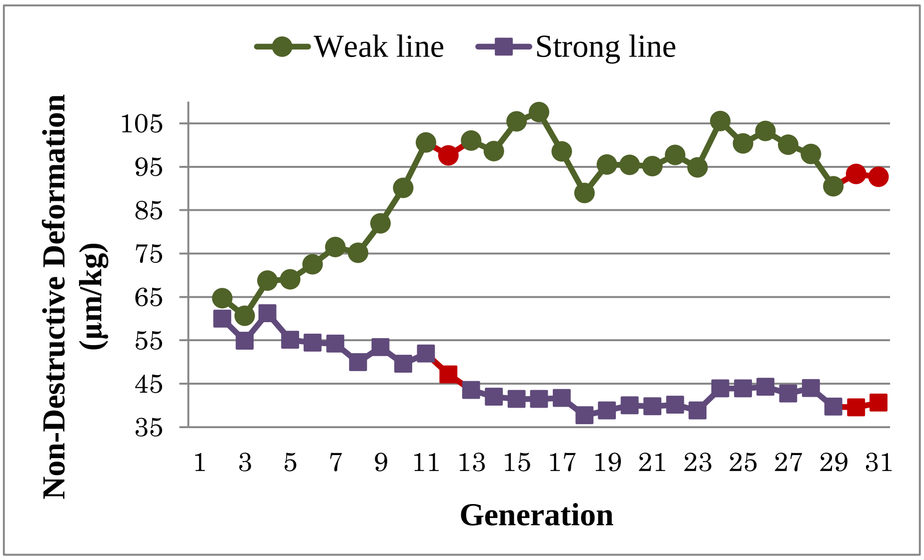

Fig. 3.13b. Generation mean for non-destructive deformation with the values obtained through multiple-regression analysis.

3.4) Relationship between traits

Now that the foundations have been laid and that the phenotypic influence of selection for non-destructive deformation on each of the traits measured has been clarified, it is possible to delve more deeply into the statistical analysis of the experiment. However, before performing a detailed analysis of the breeding values and genetic parameters, as well as examining their evolution over time, it appears relevant to review the relationship between the various phenotypic traits to gain clues as to the emergence of patterns among them. In this respect, it seems essential to examine the relationship between all of the other traits measured from two different perspectives: the first one is based on the evolution of the generation means over time, and the other one consists in looking at how the traits relate to each other within specific generations. In other words, the former denotes an *inter*-generational analysis of the phenotypic data, whereas the latter refers to an *intra*-generational analysis of the data.

3.4.1) Inter-generational analysis (all data & generation means only)

Given that this dissertation focuses on the impact of long-term selection for non-destructive deformation on three different aspects of egg quality, namely eggshell strength, egg production, and egg shape, this section is further divided into three short parts, in order to shed light on the relationship between non-destructive deformation and each aspect.

Non-destructive deformation and other eggshell traits

The relationship between non-destructive deformation and other eggshell traits is illustrated through the four scatter plots below (Figures 3.14, 3.15, 3.16, and 3.17).

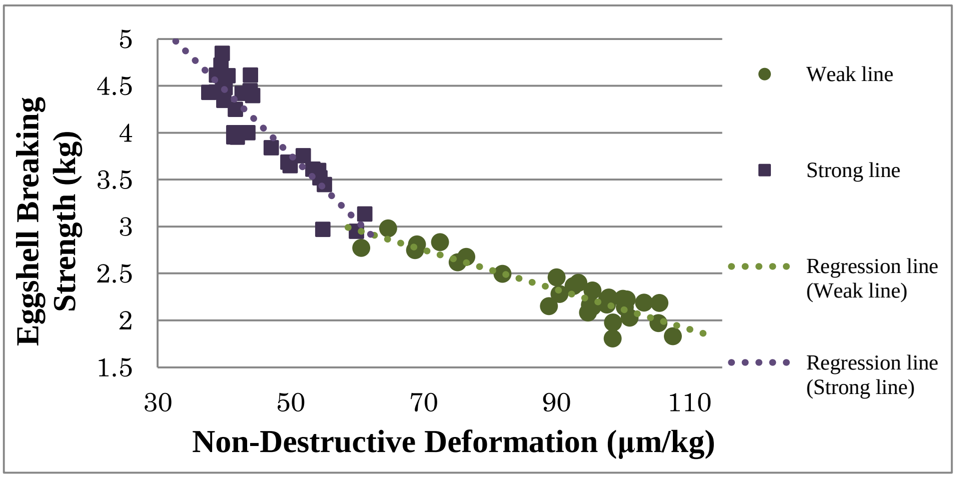

Fig. 3.14. Relationship between the generation means of non-destructive deformation and eggshell breaking strength in both lines.

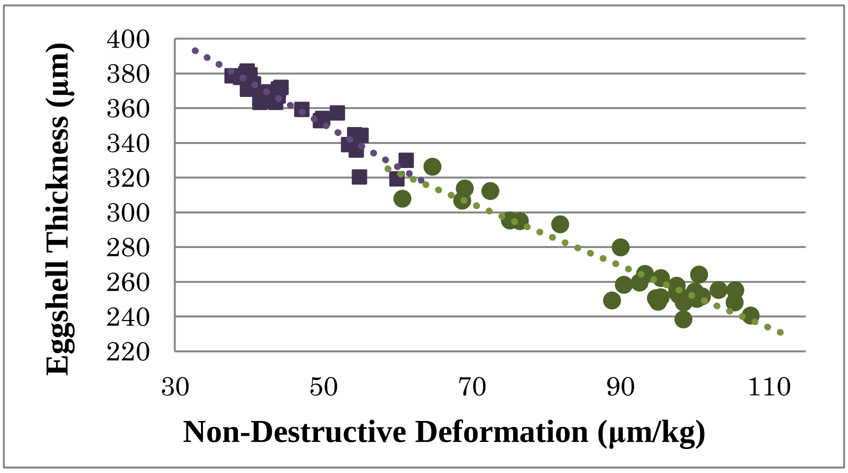

Fig. 3.15. Relationship between the generation means of non-destructive deformation and eggshell thickness in both lines.

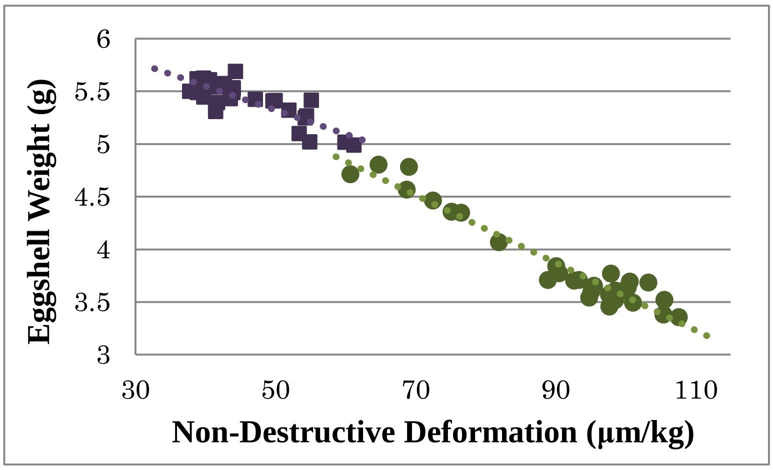

Fig. 3.16. Relationship between the generation means of non-destructive deformation and eggshell weight in both lines.

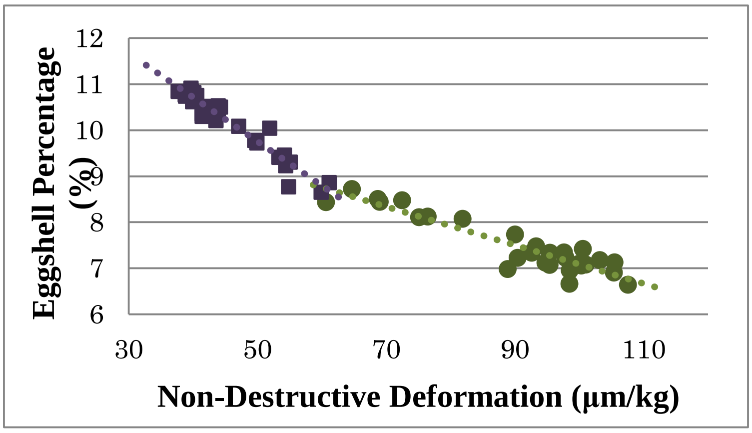

Fig. 3.17. Relationship between the generation means of non-destructive deformation and eggshell percentage in both lines.

These figures, which combine the strong and weak lines together, show that the value of non-destructive deformation tended to shape the rate of change (i.e. the regression coefficient) of the eggshell traits considered, and lead to another interesting observation: that the relationship also seemed to depend on the parameter considered. It may be so that the relationship between non-destructive deformation and is linear for some traits (as seems to be the case for eggshell thickness) and non-linear for other (for eggshell breaking strength for instance).

Non-destructive deformation and production traits

The relationship between non-destructive deformation and egg production traits was as described below (Figures 3.18, 3.19, and 3.20).

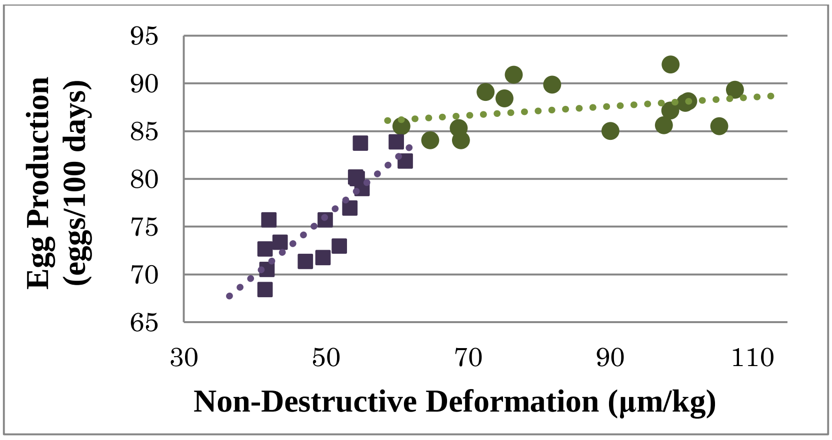

Fig. 3.18. Relationship between the generation means of non-destructive deformation and egg production in both lines.

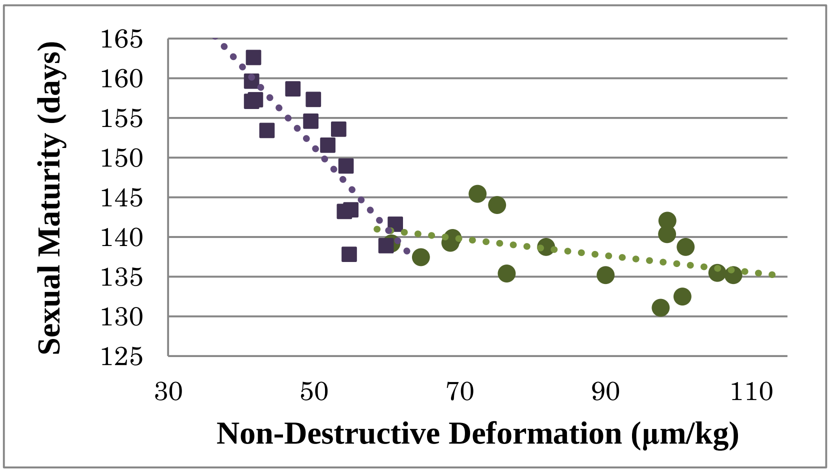

Fig. 3.19. Relationship between the generation means of non-destructive deformation and sexual maturity in both lines.

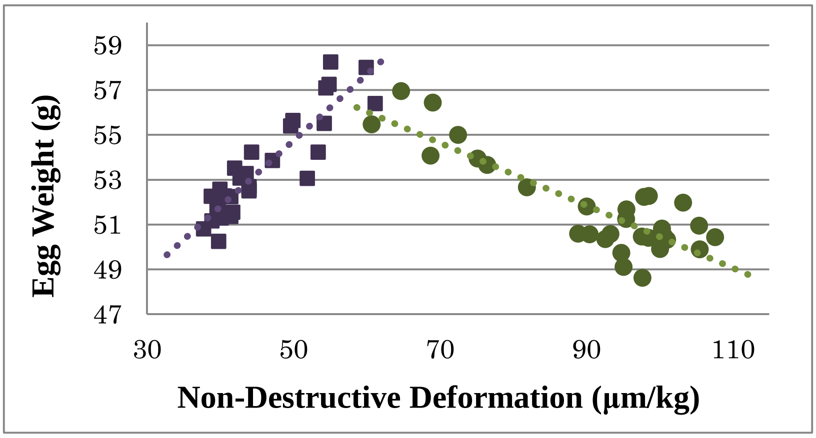

Fig. 3.20. Relationship between the generation means of non-destructive deformation and egg weight in both lines.

Based on the figures above, the relationship between eggshell strength –as expressed as a measure of the non-destructive deformation of the eggshell– and egg production traits appears to be curvilinear; the regression coefficients were indeed visibly different in the weak and strong lines. However, it may be so that specific reasons exist that explain the drop in egg weight that was observed in both lines. The detailed analysis of the following chapters will provide more clues about the underlying reasons causes for these changes.

Non-destructive deformation and egg shape

The relationship between non-destructive deformation and egg shape is illustrated through the scatter plots below (Figures 3.21, 3.22, and 3.23).

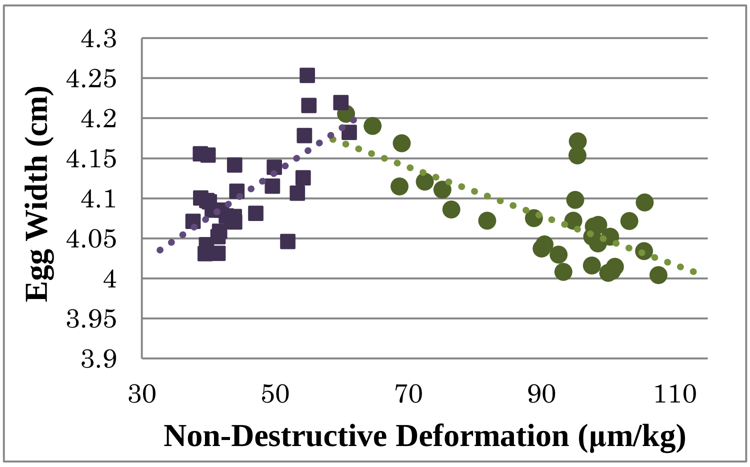

Fig. 3.21. Relationship between the generation means of non-destructive deformation and egg width in both lines.

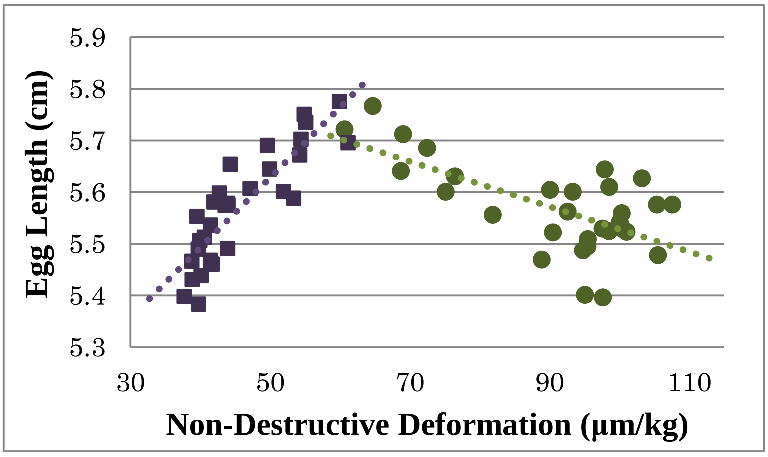

Fig. 3.22. Relationship between the generation means of non-destructive deformation and egg length in both lines.

The two graphs above, which display the relationship between non-destructive deformation and egg width (respective egg length), are similar to Figure 3.20. This is not surprising given that both egg weight and egg width (and length) are a measurement of the size of the egg.

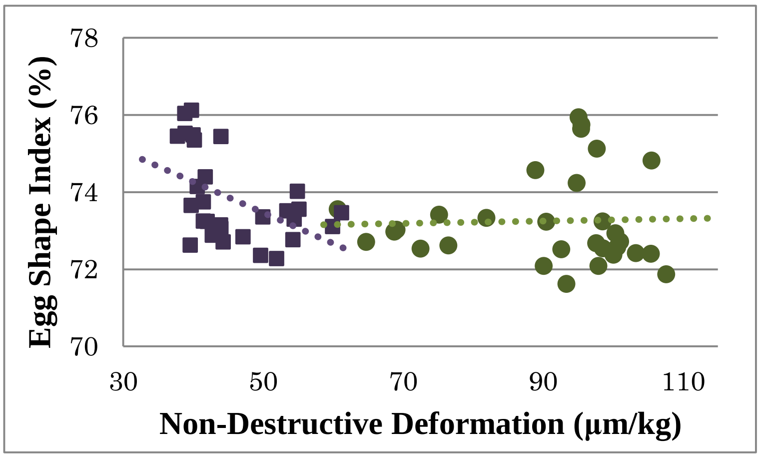

Fig. 3.23. Relationship between the generation means of non-destructive deformation and egg shape index in both lines.

In the case of egg shape index, although the phenotypic data obtained from this experiment revealed that more elongated eggs seem to be somewhat sturdier (i.e. have lower non-destructive deformation values), this relationship may not be linear. The analysis of genetic parameters will provide more information about this point as well.

3.4.2) Intra-generational analysis

One of the primary objectives of performing an intra-generational analysis of the phenotypic data is to investigate whether there are any differences in the distribution of the observations between the micro and macro levels, i.e. within generations vs. between generations.

For most traits, the relationship between two traits is the same at both levels; for instance, when observing the distribution of the generation means of non-destructive deformation with respect to eggshell thickness (as described above in Figure 3.15,), it appears that a large negative correlation exists: in other words, as non-destructive deformation values decrease, eggshell thickness values increase, and vice-versa. Unsurprisingly, this relationship between the two traits was also found within each generation.

Nevertheless, for some traits, a different trend was observed between the micro and macro levels. In the strong line, in which eggshell breaking strength increased over the course of the experiment, the generation mean of the width of the egg decreased, indicating a negative correlation between the two traits. However, within each generation, it appears that the phenotypic correlation between breaking strength and egg width is actually positive, as illustrated by Figure 3.24. This may indicate that although the correlation between the two traits is generally positive, external factor(s) have brought about a reduction in egg size over time. Additionally, the positive correlation between these two traits may suggest that greater force is required to break eggs that are wider –i.e. rounder.

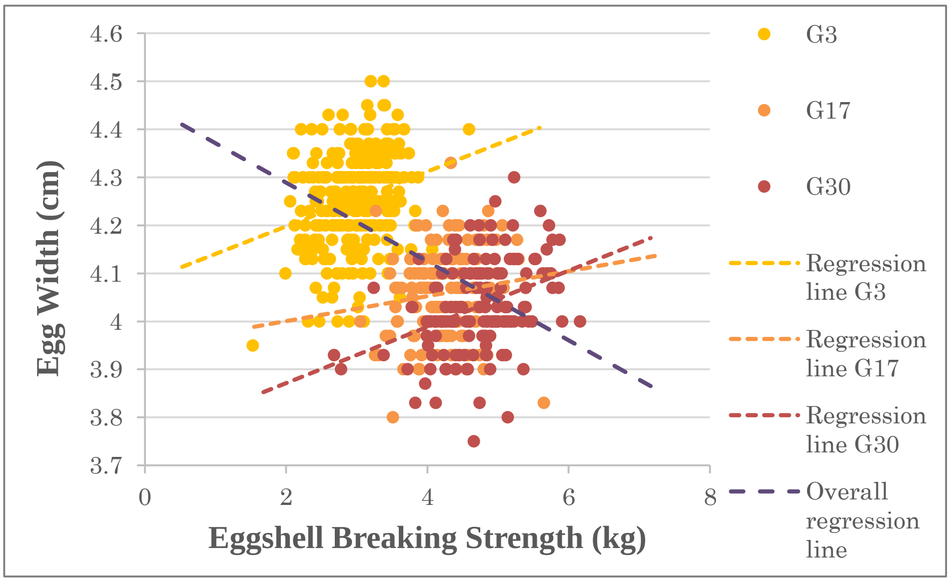

Fig. 3.24. Evolution of the generational data between eggshell breaking strength and egg width in the strong line.

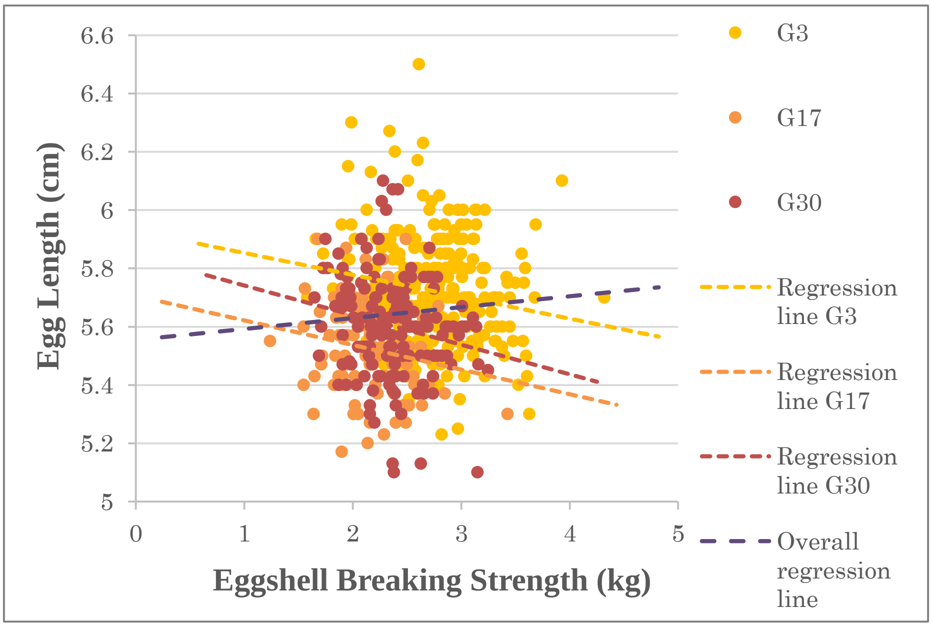

Fig. 3.25. Evolution of the generational data between eggshell breaking strength and egg length in the weak line.

A similar observation was made in the weak line, between eggshell breaking strength and egg length (Figure 3.25). At the generational level, low eggshell breaking strength values are associated with longer –i.e. more elongated– eggs. This negative correlation may indicate that elongated eggs are somewhat weaker than rounder eggs. However, over time, the average egg length decreased as breaking strength decreased. This finding tends to confirm the results and interpretation provided above for the strong line.

3.5) Realized heritability

The selection differential (cumulative), selection response (cumulative) and realized heritability for both lines are presented below (Table 3.7 and Table 3.8).

Table 3.7. Cumulative selection differential and response, and realized heritability (strong line).

Gen [1]	NDD		BS		ST	
	SD	SR	SD	SR	SD	SR
1-2	N/A	N/A	0.28	-0.03	15.5	-7.0
2-3	9.8	5.0	0.68	-0.01	36.7	-6.0
3-4	18.7	-1.3	1.05	0.15	54.8	3.6
4-5	26.9	4.8	1.35	0.47	69.0	18.0
5-6	33.5	5.5	1.73	0.54	85.8	9.5
6-7	40.7	5.7	2.09	0.62	105.1	18.3
7-8	45.9	10.0	2.32	0.67	117.3	27.7
8-9	51.9	6.5	2.64	0.63	136.1	12.6
9-10	59.1	10.3	2.99	0.70	156.2	26.5
10-11	65.1	8.0	3.36	0.78	174.1	30.9
11-12	71.0	12.8	3.67	0.86	189.7	32.9
12-13	74.5	16.4	4.12	1.02	199.3	36.9
13-14	78.4	17.9	4.38	0.97	211.1	38.4
14-15	80.2	18.5	4.49	0.97	215.9	39.5
15-16	83.1	18.5	4.65	1.02	224.0	37.0
16-17	84.9	18.2	4.76	1.27	229.5	42.8
17-18	87.0	22.2	4.90	1.45	237.2	52.3
H^2	0.255		0.295		0.220	

[1] Gen, Generation; NDD, Non-destructive deformation (µm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (µm); SD, Cumulative selection differential; SR, Cumulative selection response; H^2, Realized heritability; N/A, Not available.

Table 3.8. Cumulative selection differential and response, and realized heritability (weak line).

Gen [1]	NDD		BS		ST	
	SD	SR	SD	SR	SD	SR
1-2	N/A	N/A	0.18	0.21	8.7	18.6
2-3	9.8	-4.0	0.58	0.23	30.2	19.7
3-4	21.7	4.0	0.93	0.17	49.2	12.7
4-5	32.6	4.4	1.23	0.15	68.3	14.2
5-6	43.8	7.8	1.60	0.30	90.2	31.5
6-7	55.6	11.8	1.93	0.36	109.3	31.0
7-8	65.4	10.5	2.17	0.48	123.2	33.2
8-9	78.1	17.2	2.51	0.52	144.6	46.5
9-10	91.4	25.4	2.86	0.76	164.9	62.2
10-11	105.2	35.9	3.13	0.81	182.0	68.7
11-12	119.8	32.9	3.40	0.95	197.7	74.7
12-13	126.9	36.3	3.71	1.00	209.1	78.1
13-14	137.7	33.9	3.87	1.01	219.5	78.2
14-15	142.5	40.7	3.94	1.15	224.0	85.7
15-16	147.5	42.9	4.02	1.17	229.3	88.0
16-17	148.1	33.8	4.04	0.83	229.2	77.1
17-18	147.8	24.2	4.06	0.66	229.6	64.3
H^2	0.164		0.163		0.280	

[1] Gen, Generation; NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm); SD, Cumulative selection differential; SR, Cumulative selection response; H^2, Realized heritability; N/A, Not available.

The realized heritability for non-destructive deformation was 0.255 in the strong line and 0.164 in the weak line. These figures are based on the cumulative selection differential and response calculated up to generation 17, after which selection was ceased. It is interesting to observe that, even though selection was performed solely based on a single trait –non-destructive deformation–, the other eggshell traits also responded positively to the process. With very few exceptions, the selection differentials and responses for eggshell breaking strength and eggshell thickness were positive, and overall gave realized heritability values of 0.295 and 0.220 in the strong line, and of 0.163 and 0.280 in the weak line, respectively.

These calculations close this chapter on phenotypic data analysis, which has provided the readers with a solid general overview of the most visible outcomes of this experiment. This was achieved by using essential statistical methods, which were applied based on the data gathered. It is however necessary to go in-depth with the analysis of the variance components and other genetic parameters in order to be able to critically discuss and make relevant conclusions about the results obtained from this experiment. This will be the objective of the three following chapters, which correspond to three independent research articles published in international scientific journals. In order to remain faithful to the original articles, the text was replicated as is; only a few modifications have been made to suit the format of this dissertation.

Chapter IV

Multiple-trait analysis of a long-term selection experiment for non-destructive deformation in White Leghorns: Evolution of genetic parameters for traits related to eggshell strength

(Research Article I)

Note from the author:
This section has been abridged for copyright purposes. Please refer to the following research article for a comprehensive account of this chapter.

Gervais, O., Nirasawa, K., Vincenot, C., Nagamine, Y., & Moriya, K. (2016). Multiple-trait analysis of a long-term selection experiment for non-destructive deformation in White Leghorns: Evolution of genetic parameters for traits related to eggshell strength. *Livestock Science 189,* 56-62.
doi: 10.1016/j.livsci.2016.05.007

Summary

This chapter provides an analysis of the experiment described in the first chapters of this thesis using the REML methodology and the BLUP approach under a five-trait animal model. The five traits considered are non-destructive deformation, eggshell breaking strength, eggshell thickness, eggshell percentage, and eggshell weight, and the model also includes the generation effect as a fixed environmental effect.

The breeding values, heritabilities, genetic correlations and other genetic parameters were estimated for each line, and further analysis was conducted to examine how these parameters evolved over the course of the experiment.

Large genetic correlations were found between non-destructive deformation and the other eggshell traits, and the heritability estimates were moderately high. This indicates the potential of non-destructive deformation as a criterion to evaluate eggshell strength instead of more conventional methods, including destructive methods such as eggshell breaking strength.

Chapter V

Long-term selection using a single trait criterion, non-destructive deformation, in White Leghorns: Effect over time on genetic parameters for traits related to egg production

(Research Article II)

Note from the author:

This section has been abridged for copyright purposes. Please refer to the following research article for a comprehensive account of this chapter.

Gervais, O., Nirasawa, K., Vincenot, C., Nagamine, Y., & Moriya, K. (2016). Long-term selection using a single trait criterion, non-destructive deformation, in White Leghorns: Effect over time on genetic parameters for traits related to egg production. *Animal Science Journal xx,* xx-xx.
doi: 10.1111/asj.12653

Summary

This chapter provides an analysis of the first seventeen generations of the experiment described in the first chapters of this thesis using the REML methodology and the BLUP approach under a four-trait animal model. The following traits were considered: non-destructive deformation, egg production, egg weight, and sexual maturity. The model also includes the generation effect as a fixed environmental effect.

The breeding values, heritabilities, genetic correlations and other genetic parameters were estimated for each line, and further analysis was conducted to examine how these parameters evolved over the course of the experiment.

The selection process used in this experiment had a negative impact on egg production and sexual maturity. This suggests the need to use for instance a selection index to balance selection between eggshell and egg production traits. However, large eggs were not associated with poor eggshell quality.

Chapter VI

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

(Research Article III)

Note from the author:

This section has been abridged for copyright purposes. Please refer to the following research article for a comprehensive account of this chapter.

Gervais, O., Nirasawa, K., Vincenot, C., Nagamine, Y., & Moriya, K. (2016). Effect of long-term selection for non-destructive deformation on egg shape in White Leghorns. *Journal of Poultry Science 53*(4), xx-xx.
doi: 10.2141/jpsa.0160014

Summary

Egg geometry is an important determinant of eggshell strength. This chapter clarifies the effect of selection for non-destructive deformation on egg shape, by examining how this experiment impacted the egg shape index (the width to length ratio).

This analysis is based on the REML methodology and uses the BLUP approach under a multivariate animal model. Five traits were considered: non-destructive deformation, eggshell breaking strength, egg width, egg length, and egg shape index. The model also includes the generation effect as a fixed environmental effect. The breeding values, heritabilities, genetic correlations and other genetic parameters were estimated for each line.

This analysis revealed that selection for non-destructive deformation effectively influenced the egg shape index. The genetic correlation coefficient between eggshell breaking strength and egg shape index indicated that, for the range of values considered, rounder eggs tended to be sturdier than more elongated eggs. The results also indicate the potential to improve the shape index through genetic gain.

Chapter VII

Discussion & Conclusion

In light of the results obtained throughout the analysis of this selection experiment for non-destructive deformation, this concluding chapter is the opportunity to accomplish several important tasks. To begin with, this part provides a recapitulation of the findings presented in the three research articles that constitute the foundation of this thesis (Chapter IV, Chapter V, and Chapter VI). That summary will then be followed by a simple simulation of the economic implications of these results, which will also include a brief discussion of the usefulness of selection for a single-trait criterion in general, and for non-destructive deformation more specifically. Thirdly, several other elements that deserve more attention will be addressed in more details, such as the issues concerning the link between egg breakage and eggshell strength. This dissertation will then be concluded with a few final remarks about research prospects for the future.

7.1) Summary of findings

Overall, the selection process for non-destructive deformation was efficient: not only were the two lines created clearly divergent, but the moderately high heritability values estimated for non-destructive deformation for the entire data set, i.e. from generation 1 to generation 31, also showed that selecting for non-destructive deformation is a relevant and efficient approach to achieve the long-term genetic improvement of eggshell strength (depending on the model used, the value of the estimated heritability for non-destructive deformation for the entire data set varied from 0.381 to 0.388 in the strong line).

With respect to the first research article, which focused on eggshell traits, high genetic correlations were found between non-destructive deformation and the four other traits measured that are related to eggshell strength. Concretely, the genetic correlations fluctuated between –0.623 ± 0.036 and –0.903 ± 0.015 in the strong line and between –0.730 ± 0.027 and –0.934 ± 0.010 in the weak line. This indicates that non-destructive deformation is a good indicator of eggshell strength in general, that has the potential to replace destructive methods used in the poultry industry for eggshell strength evaluation.

In the second research article, which investigated the long-term impact of selection for non-destructive deformation on egg production traits, a statistically significant negative impact on egg production and sexual maturity was detected. It was therefore concluded that breeders should balance the selection for non-destructive deformation with that of egg production traits. At the same time, the results of the experiment also suggest that selecting for eggshell strength does not have a deleterious effect on egg size, as measured by egg weight. In other words, it appears that large egg size is not related to poor eggshell quality.

The third research paper examined the effect of long-term selection for non-destructive deformation on egg shape. This is an essential research question given that the traits related to the geometry of the egg, such as egg shape index, account for a substantial part of the variability in eggshell strength that remains after conventional factors –e.g. eggshell thickness and specific gravity– have been taken into account. In simple terms, the results showed that for the range of values studied, that is for eggs whose egg shape index lies between 71% and 76%, rounder eggs tended to be somewhat sturdier than more elongated eggs.

7.2) Assessment of economic profit

Based on the results above, it is interesting to consider the economic impact of this single-trait selection experiment for non-destructive deformation, and it is necessary to this end to explore the link between egg breakage and eggshell strength. Several studies have already tried to clarify this point, for example Ernst and Johnson (1977), who estimated that the linear relationship between egg breakage and eggshell thickness could be expressed by the following equation:

$$\hat{y} = 25.57 - 1506.4x \tag{7.1}$$

where

$\hat{y}$ represents egg breakage (in %), and

x is the thickness of the eggshell (in inches)

In the experiment analyzed throughout the current dissertation, the mean value of eggshell thickness for generation 1 was 326.4 µm (cf. Chapter III, Table 3.1). After converting to the relevant unit (inches), a simple calculation shows that this value corresponds to an estimated breakage of 6.2%, according to the equation above. In Chapter IV, it was found that in the strong line, the breeding values for eggshell thickness had increased by 61.3 µm (+18.8%) by generation 17, when selection was stopped. This means that the individuals of generation 17 in the strong line were expected to have offspring whose eggs are 387.7 µm thick on average (since 326.4 + 61.3 = 387.7). Using the same equation, this new value corresponds to an estimated breakage rate of 2.2%. Compared to the breakage percentage calculated for generation 1, this translates into an extra 4 eggs reaching the consumer per 100 eggs for the offspring of generation 17.

On the downside, it was revealed in Chapter V that the egg production had decreased as a consequence of the selection process for non-destructive deformation. Although initially the generation mean was 87.0 eggs laid per 100 days for generation 1, a detailed analysis revealed that the breeding values had indeed decreased by 7.6 eggs laid per 100 days by generation 17 in the strong line. This 8.5% decrease in egg production therefore completely offsets the 4% increase in the number of eggs reaching the consumer that results from improved eggshell quality, even before accounting for the delay of the onset of sexual maturity also detected.

From this simple calculation, it appears that selection for a single trait does not improve shell quality sufficiently to make up for the negative impact estimated on the traits related to egg production, thereby leading to the conclusion that the best alternative for breeders is to introduce a selection index that balances the selection for eggshell traits with that of egg production traits.

At the same time, in practice the comparison made above between the 4% increase in the number of eggs reaching the consumers and the 8.5% decrease in egg production is unlikely to translate exactly into a 4.5% decrease overall. As a matter of fact, while in Chapter I the percentage of eggs lost because of poor eggshell quality was first calculated, and the total cost for the poultry industry was subsequently inferred based on the price of an egg or of a dozen, it is hard to accurately assess the exact cost for the producers of each additional cracked or broken egg in the system. For instance, depending on the stage of egg processing, a broken egg may only represent the loss of one egg, but it may also soil an entire dozen. For this reason, with all other conditions being equal, a decrease of the number of broken eggs by X eggs is likely to be more profitable than an increase in production by X eggs, from an economic perspective.

Such factors make the accurate estimation of the financial cost of egg breakage difficult, especially given that a variety of egg processing systems exist. Nevertheless, further research into this question would allow scholars and breeders to better quantify the actual economic benefits resulting from the improvement of eggshell strength.

7.3) Further discussion and concluding remarks

With regard to the relationship between egg breakage and non-destructive deformation, additional observations are necessary as a complement to the analysis made so far. As noted by Hamilton *et al.* (1979), the types of external stress to which eggshells are exposed in the field can be divided into two categories: the shocks caused by impact –i.e. the collision of the egg with another egg or object– and the strain on the eggshell that ensues from compression on the surface of the egg, such as when the eggs are stacked. Given the different nature of these forces, it is important to test for both types of external stress to ensure the quality of the egg. In this respect, using non-destructive deformation is only useful to measure the tolerance of the eggshell to compression forces. It is therefore crucial to also implement at least one method to test for the tolerance of the eggshell to accidental impacts.

Although these considerations do not alter the relevance of this dissertation, this point is of particular importance in light of the fact that eggs are exposed to these two types of external stress at different stages of the egg processing system. Identifying how each step of the process affects the eggshell therefore provides further insight about when increased shell strength –as measured by non-destructive deformation– is actually useful in the production chain. In this respect, Orr *et al.* (1977) reported that compression forces are more likely to lead to cracks or breakage during shipping, washing, grading, and packing. This means that although improvements in non-destructive deformation are very likely to induce a significant decrease in the rate of breakage during these stages, the increased strength may not influence as much the other steps of the production process, such as the initial gathering of the eggs.

Before concluding this discussion about egg breakage in the field, it is important to mention that in practice the eggs may be subject to compression forces at any point on the surface of the eggshell, but that researchers conventionally measure non-destructive deformation on the minor axis of the egg rather than on the major axis, which is significantly stronger. The reason for this practice lies in the fact that the measurements on the minor axis are generally more reliable when it comes to predicting fracture force. Indeed, experimental data shows that a quite large amount of the eggs whose deformation was measured on the major axis crack at the smaller pole, which is supposedly stronger (Voisey & Hamilton, 1976). This reduces the correlation with factors such as breaking strength. On a similar note, Voisey & Hamilton (1976) also showed that 1 kg is the value for which the measurement of non-destructive deformation best predicts the breaking strength of the egg.

Overall, the analysis conducted throughout this thesis showed both the potential and the limitations of using non-destructive deformation as a single criterion for selection. It would be interesting to perform a long-term selection experiment for non-destructive deformation that uses a selection index including egg production traits, and compare the results with the findings presented in this paper.

Another interesting aspect that needs to be explored is the impact of long-term selection for non-destructive deformation on the internal quality of the egg. Although the introducing chapter of this dissertation mentions the fact that this aspect lies beyond the scope of this study, it does not mean that it is irrelevant. For instance, eggshell thickness is closely related to the moisture level inside of the egg, with thicker eggshells losing less moisture in comparison with eggs whose shell is thinner. Greater shell thickness is therefore a factor that tends to slow down the deterioration process, thereby allowing to preserve the eggs longer, and it also increases hatching (Rogue & Soares, 1994). Such effects need to be better understood and quantified to prevent contamination, minimize health risks, and enhance food security in the future.

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Appendix

Appendix 1a. Phenotypic description (mean ± standard deviation) of the strong line, according to trait and generation. The strong line refers to the line that was selected for decreased non-destructive deformation value and therefore has stronger eggshells.

Gen	n	NDD	EP	EW	SM
1	412	N/A	87.0 ± 8.0	58.6 ± 3.9	143.8 ± 9.8
2	284	59.9 ± 10.4	83.9 ± 8.7	58.0 ± 3.9	138.9 ± 9.2
3	275	54.9 ± 9.5	83.7 ± 9.9	57.3 ± 3.8	137.8 ± 10.6
4	266	61.2 ± 8.3	81.9 ± 7.3	56.4 ± 3.6	141.6 ± 10.3
5	301	55.1 ± 7.3	79.0 ± 7.9	58.3 ± 3.3	143.4 ± 8.0
6	297	54.4 ± 7.0	80.0 ± 7.7	57.1 ± 3.9	149.0 ± 8.8
7	290	54.2 ± 7.2	80.2 ± 6.7	55.5 ± 3.4	143.2 ± 11.7
8	288	49.9 ± 5.6	75.7 ± 7.5	55.6 ± 3.7	157.3 ± 10.8
9	292	53.4 ± 7.1	77.0 ± 8.5	54.2 ± 2.9	153.6 ± 10.5
10	288	49.6 ± 6.7	71.8 ± 10.2	55.4 ± 3.8	154.6 ± 15.2
11	248	51.9 ± 6.2	73.0 ± 9.5	53.1 ± 3.2	151.6 ± 13.0
12	254	47.1 ± 5.1[a]	71.4 ± 9.2	53.9 ± 3.0	158.7 ± 15.2
13	198	43.6 ± 5.2	73.4 ± 10.0	53.3 ± 2.9	153.4 ± 10.3
14	130	42.0 ± 4.3	75.7 ± 7.7	53.5 ± 3.1	157.3 ± 9.2
15	136	41.4 ± 6.3	68.4 ± 11.4	52.3 ± 2.6	159.6 ± 10.8
16	146	41.4 ± 4.7	72.7 ± 7.8	51.4 ± 3.0	157.1 ± 12.4
17	150	41.7 ± 5.0	70.5 ± 11.8	51.5 ± 2.9	162.6 ± 14.7
Total	**4255**				

EP, egg production; EW, egg weight; Gen, generation; n, number of records (hens); N/A, not available; NDD, non-destructive deformation; SM, sexual maturity.

[a] The value for non-destructive deformation for generation 12 was calculated by using multiple-regression analysis, according to the other eggshell traits.

Appendix 1b. Phenotypic description (mean ± standard deviation) of the weak line, according to trait and generation. The weak line refers to the line that was selected for increased non-destructive deformation value and therefore has weaker eggshells.

Gen	n	NDD	EP	EW	SM
1	412	N/A	87.0 ± 8.0	58.6 ± 3.9	143.8 ± 9.8
2	300	64.7 ± 11.8	84.0 ± 9.2	57.0 ± 3.7	137.5 ± 8.1
3	268	60.7 ± 10.9	85.5 ± 10.2	55.5 ± 3.7	139.2 ± 9.2
4	258	68.7 ± 9.9	85.3 ± 8.5	54.1 ± 3.5	139.3 ± 9.0
5	280	69.0 ± 10.0	84.1 ± 10.3	56.4 ± 3.8	139.9 ± 10.9
6	291	72.5 ± 11.2	89.1 ± 10.0	55.0 ± 3.4	145.4 ± 10.9
7	271	76.5 ± 11.1	90.9 ± 7.4	53.7 ± 3.4	135.4 ± 9.9
8	265	75.1 ± 10.6	88.4 ± 8.9	53.9 ± 3.3	144.0 ± 8.6
9	283	81.9 ± 11.9	89.9 ± 9.5	52.7 ± 3.3	138.7 ± 9.7
10	277	90.1 ± 12.0	85.0 ± 8.9	51.8 ± 3.2	135.2 ± 6.2
11	250	100.6 ± 13.4	88.0 ± 8.6	50.5 ± 3.3	132.5 ± 7.0
12	250	97.6 ± 8.6[a]	85.6 ± 10.7	50.5 ± 3.0	131.1 ± 7.6
13	187	101.0 ± 12.2	88.1 ± 8.1	50.3 ± 2.7	138.7 ± 10.5
14	136	98.5 ± 12.3	92.0 ± 6.6	52.3 ± 3.1	142.0 ± 8.6
15	129	105.4 ± 13.9	85.5 ± 9.2	51.0 ± 3.5	135.5 ± 9.5
16	140	107.6 ± 16.4	89.3 ± 8.3	50.4 ± 3.1	135.2 ± 11.9
17	97	98.5 ± 15.2	87.1 ± 12.5	50.4 ± 2.8	140.4 ± 11.3
Total	**4094**				

EP, egg production; EW, egg weight; Gen, generation; n, number of records (hens); N/A, not available; NDD, non-destructive deformation; SM, sexual maturity.

[a] The value for non-destructive deformation for generation 12 was calculated by using multiple-regression analysis, according to the other eggshell traits.