

Segregation analysis of blood oxygen saturation in broilers suggests a major gene influence on ascites

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Abstract 1. Blood oxygen saturation (SaO) is a potential indicator trait for resistance to ascites in chickens.
2. The objective of the study was to investigate the genetic architecture of SaO in a meat-type chicken line reared in commercial conditions.
3. Data were collected over 15 generations of selection and were divided into two data sets on the basis of a change in recording age from 6 to 5 weeks of age, approximately halfway through the period. The resulting pedigrees comprised in excess of 90 000 birds each and, on average, 12% of these birds had SaO records.
4. Segregation analyses of SaO were carried out assuming a mixed inheritance model that included a major locus segregating in a polygenic background.
5. The analyses suggest that a major gene is involved in the genetic control of SaO in this line. The putative gene acts in a dominant fashion and has an additive effect of around $0.90 \sigma_p$, equivalent to a predicted difference in SaO between the two homozygous classes of more than 10%. The frequency of the allele that increases SaO changed from 0.53 to 0.65 from the first to the second set of data, consistent with selection on SaO scores.
6. Using estimated genotype probabilities at the putative major locus, we inferred that it acts in an overdominant fashion on body weight and fleshing score. If the low SaO allele leads to susceptibility to ascites, its combined effects are consistent with it being maintained in the population by a balance of natural selection on fitness and artificial selection on growth and carcass traits.
7. Even with selection on both SaO and growth traits, the combined genotypic effects would make it difficult to remove the unfavourable low-SaO allele by means of traditional selection without the use of genetic markers.

Abbreviation Key: a = additive effect; d = dominance effect; Flesh = fleshing score; MCMC = Markov Chain Monte Carlo; p_b = frequency of the *b* allele; p_B = frequency of the *B* allele; p_{bb} = frequency of the *bb* genotype; p_{Bb} = frequency of the *Bb* genotype; p_{BB} = frequency of the *BB* genotype; REML = restricted maximum likelihood; RN = Napole yield; SaO = blood oxygen saturation; Weight = body weight; σ_e^2 = residual variance; σ_m^2 = major locus variance; σ_p^2 = phenotypic variance; σ_u^2 = polygenic variance;

INTRODUCTION

Blood oxygen saturation (SaO) is a potential indicator trait for resistance to ascites in chickens (Wideman *et al.*, 1998). In a previous study (Navarro *et al.*, 2006), we presented heritabilities

of SaO, body weight (Weight) and fleshing score (Flesh, a measure of breast conformation), and genetic correlations between these traits for four meat-type chicken lines. We showed that SaO was moderately heritable (heritabilities for this trait ranged from 0.1 to 0.2) and that its genetic

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correlation with production traits was low, so that simultaneous selection for increased SaO and increased production should be possible. These genetic parameters were obtained assuming an infinitesimal model (Fisher, 1918), which assumes that the (quantitative) trait is influenced by an infinite number of unlinked loci (polygenes), each with an infinitely small additive effect on the trait. However, in recent years, several studies have shown that one or few genes, or quantitative trait loci (QTL), explain an important proportion of the phenotypic or genetic variation for some quantitative traits. Today, examples of genes or QTL with large effect on quantitative traits of agricultural interest are numerous. For example, the RN-mutation in pigs (Milan *et al.*, 2000) affecting meat quality, the Booroola gene (Piper and Bindon, 1982) in sheep affecting ovulation rate and the double muscling gene (Hanset and Michaux, 1985*a,b*) in cattle.

Several of the known major genes in livestock were first inferred from their effects on segregation of phenotypes within pedigrees. Although simple metrics can provide evidence for major gene segregation, fullest use of available data is made in the context of an appropriate analytical model. Mixed inheritance models were first introduced in human genetics to discriminate between modes of inheritance and particularly to infer the presence of major genes (Elston and Stewart, 1971; Morton and MacLean, 1974). These methods were first used within a maximum likelihood framework, which restricted their practical use to the analysis of small pedigrees. Although a series of approximations of the mixed inheritance model likelihood are available for animal breeding populations with simple structures (see, for example, Knott *et al.*, 1992), analysis of large complex pedigrees (like the ones usually encountered in animal breeding) is only feasible when sampling based techniques are used either to estimate likelihoods (Guo and Thompson, 1991, 1994) or to implement Bayesian analysis (Janss *et al.*, 1995).

Markov Chain Monte Carlo (MCMC) sampling methods provide an efficient means to carry out these tasks. In particular the Gibbs sampler, an MCMC method, is now widely used in genetic analyses. It is capable of generating samples from the joint distribution (usually complex) of several random variables by sampling from known and simple conditional distributions. From these samples, marginal distributions of each variable can be obtained and provide estimates of the posterior distributions of the model parameters.

Little is known about the genetics underlying ascites-related traits. Here, we investigate the genetic architecture of SaO using segregation

analysis. Data come from the line for which we obtained the highest estimates of genetic variance and heritability for SaO in our previous analyses (Navarro *et al.*, 2006). This line shows a slightly higher ascites-related mortality than other Aviagen Ltd. lines (A. Koerhuis, personal communication) and it is the heaviest of the lines studied. We study the possible existence of a major gene or quantitative trait locus (QTL) involved in the control of SaO. To this end, we have carried our analyses assuming a mixed inheritance model that includes a major locus as well as polygenes. In subsequent analyses, we have used the estimated genotype probabilities at the putative major locus to investigate its effect on body weight and fleshing score. The results obtained are consistent with the segregation of a major gene and help reconcile observations on the genetic correlations between traits, the incidence of ascites and its relationship with selection on growth and carcass traits.

MATERIALS AND METHODS

Data

Two data sets (data sets 1 and 2) that each consisted of around eight overlapping generations of selection were available from a meat-type chicken population. The population studied is a male line that has been closed for between 30 and 40 generations and has a history of selection on wheat diets. Selection has been more focused on growth rate and efficiency and less emphasis has been placed on yield. In addition the population has been exposed to considerable family selection against broiler mortality and leg defects. Both data sets contained records of SaO, body weight (Weight) and fleshing score (Flesh, a measure of breast conformation). SaO was directly measured using a pulse oximeter. A sensor was attached to a Criticare Pulse Oximeter 504US (Criticare Systems Inc., Milwaukee, USA, supplied by R.L. Dolby, Stirling, UK) and positioned on the wing to illuminate the tissue between the radius and ulna for measurement of percentage saturation of haemoglobin with oxygen (Julian and Mirsalimi, 1992). When the bird was settled, two readings were taken, and the record for this bird was taken to be an average of both readings. Fleshing is a conformation score with higher scores relating to superior breast muscle development relative to the size of the whole bird. Recording was carried out in handheld terminals grading the birds between 1 and 5, 1 being the birds with the least breast meat and 5 the most. Feel of the breast breadth and depth and length of the keel were also taken into account. SaO data were only available for male selection candidates whereas

Table 1. Structure of data for oxygen saturation (SaO), body weight (Weight) and fleshing score (Flesh) for data sets 1 and 2

Category	SaO		Weight and Flesh	
	Data set 1	Data set 2	Data set 1	Data set 2
Birds with records	11919	13450	118782	91761
Sires/paternal half-sib families with records	667	510	755	554
Dams with progeny with records	3548	3137	4809	4004
Full-sib families with records for SaO	4130	-	4130	-

Table 2. Means and standard deviations (in brackets) for blood oxygen saturation (SaO, in% units), body weight (Weight, in kg) and fleshing score (Flesh, a measure of breast conformation, in arbitrary units, measured in a scale of 1 to 5) for raw phenotypes (RAW) and for SaO phenotypes adjusted for fixed effects (ADJ) for data sets 1 and 2. Skewness (Sk) and kurtosis (Ku) coefficients of the distributions of raw SaO phenotypes and analysed data are also presented

Data set		SaO (%)	Weight (kg)	Flesh (units)	Sk	Ku
1	RAW	80.02 (9.15)	2.72 (0.34)	3.17 (0.92)	-0.78	0.46
	ADJ	0.64 (8.08)	-	-	-0.75	1.06
2	RAW	81.81 (7.98)	2.16 (0.28)	3.10 (0.90)	-0.85	0.88
	ADJ	-0.42 (7.23)	-	-	-0.74	0.93

Weight and Flesh were recorded on most birds. Records were taken at 6 weeks of age for data set 1. Data set 2 consisted of data from the same line immediately following a shift of the recording age from 6 to 5 weeks of age.

A description of the pedigree and data structure for the data sets used is presented in Table 1. For both data sets, the mean number of birds with SaO record per full-sib family was less than 5 (with a minimum value of one and a maximum value of 25) and the mean paternal half-sib family size was less than 30 (with a minimum value of one and a maximum value of 106).

Statistical analyses

Segregation analyses to assess the possibility that a locus with large effect was involved in the genetic control of SaO were performed on data sets 1 and 2. Since results from more recent data are of greatest interest if follow-up studies were to be carried out or our findings were to have an impact on breeding programme decisions, a more comprehensive set of analyses was performed on data set 2. Details of the analyses methods are presented as an annex.

In brief, a mixed inheritance model was used for the segregation analysis. Mixed inheritance models are a combination of infinitesimal and finite gene models. In our analyses, a single major locus was modelled in addition to a polygenic effect. The major locus was assumed to be autosomal and biallelic with Mendelian transmission probabilities and with an additive (*a*) and a dominance effect (*d*). The genotypic value for birds with genotype *BB* at the major

locus is *a*, *-a* for *bb* birds and *d* for *Bb* birds. The major locus was assumed to be in Hardy-Weinberg equilibrium proportions in the 'base generation' (that is, the first generation of a data set).

These analyses provide as output distributions of the parameters estimated and the mean and the standard deviation of these distributions can be used as estimates of the parameters and their standard error. In this case, we obtained estimates of major locus effects and allelic frequencies, polygenic and residual variances, as well as genotypic probabilities for each bird. Major locus effects and frequencies are used to estimate the variance explained by the major locus, and this is then used to assess if it is likely that a major locus is indeed segregating for SaO.

We also carried out some exploratory analyses on genotype probabilities estimated for sires and selection candidates from data set 2, and investigated the effect of the putative major locus on body weight and fleshing score.

RESULTS

Data

Table 2 shows descriptive statistics of the distributions of SaO, Weight and Flesh. Means and standard deviations were obtained with GENSTAT (GENSTAT 5 Committee, 1993). Skewness and kurtosis coefficients were obtained with MINITAB 12 (MINITAB Inc., 1998). Whilst mean body weight for data set 1 was 20% higher than mean data set 2 body weight, differences were smaller for mean SaO and Flesh (respectively, 2% lower and 2% higher for data taken at

Table 3. Heritabilities (on diagonal) and genetic correlations (below diagonal) and their standard errors (in brackets), obtained for body weight (Weight), fleshing score (Flesh, a measure of breast conformation) and blood oxygen saturation (SaO) estimated by REML from data set 1 (traits measured at 6 weeks) and data set 2 (traits measured at 5 weeks) using full pedigrees

Data set		SaO	Weight	Flesh
1	SaO	0.21 (0.02)		
	Weight	-0.02 (0.06)	0.26 (0.01)	
	Flesh	-0.10 (0.05)	0.53 (0.03)	0.22 (0.01)
2	SaO	0.15 (0.02)		
	Weight	-0.10 (0.06)	0.32 (0.01)	
	Flesh	-0.10 (0.06)	0.62 (0.02)	0.19 (0.01)

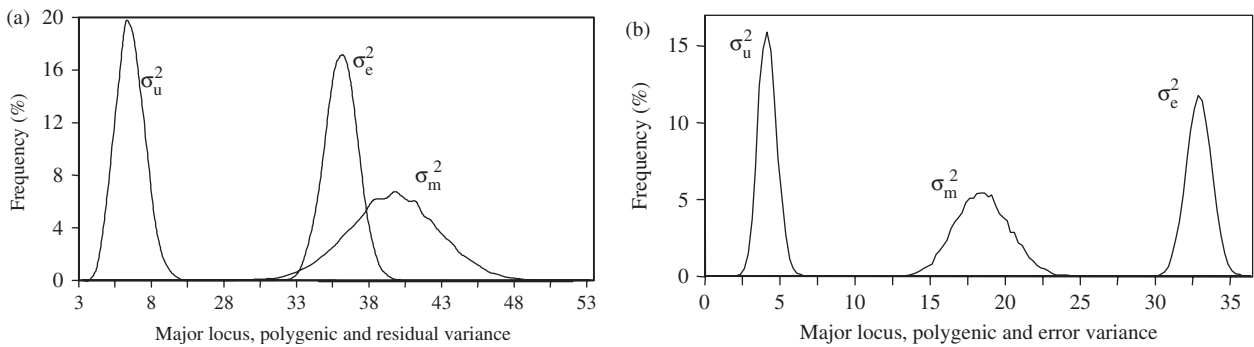


Figure 1. Pooled posterior distributions of the polygenic (σ_u^2), major locus (σ_m^2) and residual (σ_e^2) variances obtained from data sets 1(a) and 2(b).

6 weeks in the less recent pedigree). Coefficients of variation were similar within traits across data sets. SaO distributions (adjusted for fixed effects or unadjusted) showed skewness coefficients that were more extreme than -0.70 for both data sets. Adjusting raw data for fixed effects slightly decreased absolute skewness but increased kurtosis (making the distribution more leptokurtic), more markedly so for data set 1.

Table 3 shows REML estimates of heritabilities and genetic correlations (and standard errors) for SaO, Weight and Flesh obtained from trivariate analyses for data sets 1 and 2 when fitting a purely polygenic model. The estimated heritability for SaO from data set 1 was higher than that from data set 2. This was due to a 43% decrease in σ_u^2 (that changed from 14.37 to 8.18) accompanied by a smaller (16%) decrease in σ_e^2 (that changed from 54.06 to 45.18). Genetic correlations between SaO and Weight and Flesh were negative but not significantly different from 0 ($P > 0.05$) for either data set.

Segregation analyses

Figure 1 shows the pooled posterior distributions of σ_u^2 , σ_m^2 and σ_e^2 for data sets 1 and 2. Following the criteria detailed in the annex (the major locus variance distributions presented zero densities for values equal to zero) we inferred that a major locus involved in the control of SaO was

segregating in the population studied. Table 4 shows estimates and standard deviations of the major locus additive and dominance effects, B allele frequency and residual, polygenic and major locus variances as well as polygenic and major locus heritabilities and dominance deviance. The dominance deviance was very close to 1 for both data sets (1.12 ± 0.06 and 1.02 ± 0.06 , respectively) and therefore we will assume in the following that a and d can be considered equal, so that the locus acts in a dominant fashion. For data set 1 $a = d = 7.2\%$ SaO which means that the difference between bb birds and BB or Bb birds in SaO would be around 14%. The standardised locus additive effect was $0.80 \sigma_p$ (or $1.12 \sqrt{(\sigma_u^2 + \sigma_e^2)}$ or $1.21 \sigma_e$). The frequency of the major locus allele that increases SaO was estimated to be $p_B = 0.53$. This locus alone would explain 48% of the total variance and 87% of the total genetic variance in SaO. The additive genetic variance accounted for by the major locus would be 79% of the total additive genetic variance.

Figures 2 and 3 show, respectively, the distributions of a and d and of p_B for data sets 1 and 2. The estimated locus effects obtained from the analysis of data set 2 were roughly the same, although slightly smaller, than the estimates obtained from the analysis of data set 1: the estimate of a from data set 2 was proportionately 0.89 the estimate from data set 1 and the

Table 4. Point estimates and standard deviations (in brackets) of the major locus additive (a) and dominance (d) effect, B allele frequency (p_B), population mean (Mean) and residual (σ_e²) and polygenic (σ_u²) variances obtained from the segregation analysis of SaO for data sets 1 and 2. Estimates are also presented for the major locus variance (σ_m²), total phenotypic variance (σ_p²), variance ratios (h_T, h_m, h_{am} and h) and dominance deviance (d/a)

Data set	a	d	p _B	Mean	σ _e ²	σ _u ²	σ _m ²	σ _p ²	h _T	h _m	h _{am}	h	d/a
1	7.22 (0.20)	8.05 (0.32)	0.53 (0.03)	-5.60 (0.33)	35.58 (1.14)	6.06 (1.00)	39.19 (3.10)	80.83 (3.07)	0.56 (0.02)	0.48 (0.02)	0.36 (0.03)	0.14 (0.02)	1.12 (0.06)
2	6.46 (0.21)	6.60 (0.32)	0.65 (0.02)	-4.84 (0.26)	32.67 (0.85)	3.93 (0.62)	18.25 (1.82)	54.85 (1.84)	0.40 (0.02)	0.33 (0.02)	0.17 (0.02)	0.11 (0.02)	1.02 (0.06)

$$\sigma_m^2 = 2 p_B(1 - p_B) [a + d((1 - p_B) - p_B)]^2 + [2 p_B (1 - p_B) d]^2$$

$$\sigma_p^2 = \sigma_m^2 + \sigma_u^2 + \sigma_e^2$$

$$h_T = (\sigma_m^2 + \sigma_u^2) / \sigma_p^2$$

$$h_m = \sigma_m^2 / \sigma_p^2$$

$$h_{am} = 2 p_B(1 - p_B) [a + d((1 - p_B) - p_B)]^2 / \sigma_p^2$$

$$h = \sigma_u^2 / (\sigma_u^2 + \sigma_e^2)$$

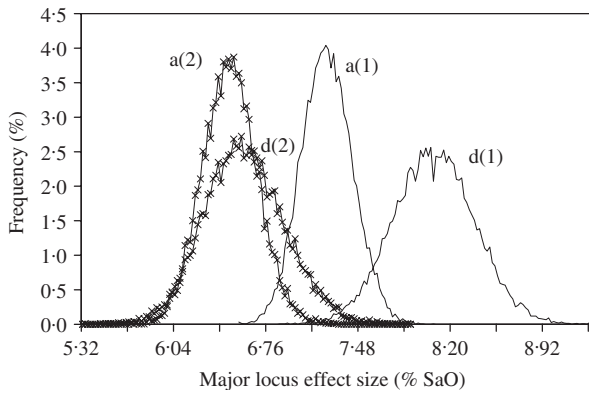


Figure 2. Pooled posterior distributions of the major locus additive and dominance effects obtained from data set 1 (a(1) and d(1)) and from data set 2 (a(2) and d(2)).

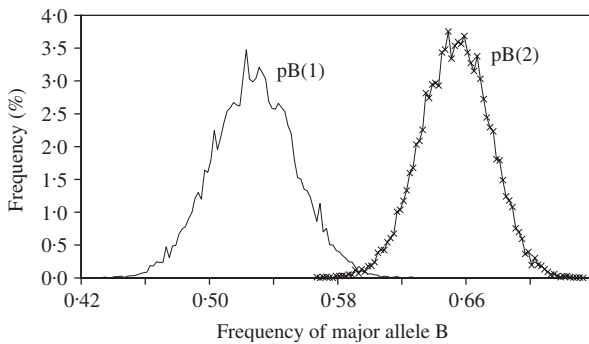


Figure 3. Pooled posterior distributions of the major allele B frequency obtained from data set 1 ($p_B(1)$) and from data set 2 ($p_B(2)$).

estimated d was 0.82 the estimate from data set 1. Despite this, the standardised locus additive effect increased from 0.80 to 0.87 σ_p . The estimated p_B was 0.65, that is, 0.12 higher than the estimate obtained from data set 1. This major locus would explain 33% of the total variance observed for SaO in data set 2 and 82% of the total genetic variance. The additive genetic variance accounted for by the major locus was estimated to be 70% of the total additive genetic variance.

Sampled genotype probabilities and allele frequencies

The correlation between chains of estimated probabilities for each genotype for individual birds was higher than 0.96, and the mean standard error (averaging over all birds with data and ancestors) of each genotype probability was smaller than 0.01.

Sire genotype probabilities obtained from data set 2 show that approximately 1.8% of the sires were assigned a bb genotype, 32.9% a Bb

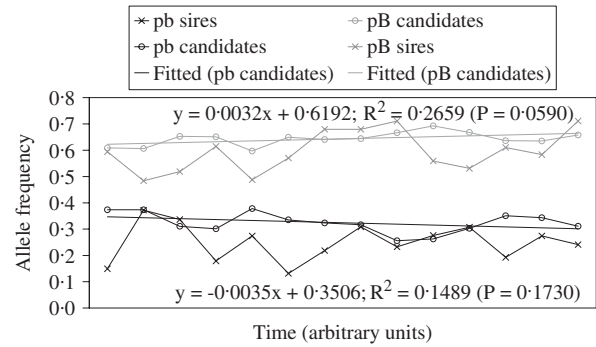


Figure 4. Major allele frequencies plotted over time. Estimates of allele frequencies were obtained from sires alone and from all birds with records.

genotype and 11.1% a BB genotype with a probability higher than 0.8. If the inferred mode of action of the putative gene is dominant, information from relatives is necessary to discriminate heterozygotes from the dominant homozygotes. In our case, more than 40% of sires were identified as being either BB or Bb with high probability (>0.8) but around further 15% of sires had similar (and close to 0.5) probabilities of being BB or Bb , although they had very low probabilities of being bb .

Figure 4 shows how major allele frequencies vary over time for data set 2. Estimates of allele frequencies were obtained from sires alone and from all birds with records. For sires, each point in the graph was obtained from 29 birds, whereas in the case of selection candidates each point was obtained from 1000 birds. For selection candidates, regression of allele frequencies over time has a negative slope for p_b and a positive one for p_B , showing how within the period corresponding to data set 2 the frequency of the major allele that increases SaO increased over time.

Estimation of the putative locus effect on weight and fleshing score

Table 5 shows the estimates of the putative locus additive and dominance effect on SaO, Weight and Flesh obtained from the regression of trait values on genotype probabilities at the major locus using data set 2. The estimated additive effect was not different from zero for Weight and Flesh, but the estimated dominance effect was large for both. This suggests that birds heterozygous at this putative locus would have substantially higher body weight and fleshing score at 5 weeks than either of the homozygotes. The estimated effects for SaO were significantly

Table 5. Estimates and standard errors (in brackets) of the putative locus additive (a) and dominance (d) effect for blood oxygen saturation (SaO), body weight (Weight) and fleshing score (Flesh, a measure of breast conformation) obtained from the regression of trait values on genotype probabilities at the major locus

Trait ¹	a	d
SaO (%)	11.96 (0.14)**	16.61 (0.27)**
Weight (kg)	-0.71×10^{-2} (0.55×10^{-2})	5.84×10^{-2} (0.88×10^{-2})**
Flesh (units)	-0.03 (0.02)	0.24 (0.04)*

¹ Data recorded at 5 weeks of age (data set 2) were used to obtain these estimates.

*Significantly different from 0 ($P \leq 0.05$).

**Significantly different from 0 ($P \leq 0.01$).

larger than the estimates obtained from the segregation analysis.

DISCUSSION

Overall, the segregation analyses of SaO data indicate that a locus with large effect is involved in the genetic control of SaO in this line. Results obtained from the two sets of data provide a consistent picture: a dominant major locus with an additive effect of around $0.90 \sigma_p$ that is responsible for a predicted difference in SaO between the two homozygotes of more than 10% is segregating in this line. The frequency of the allele that increases SaO increased with selection on SaO from $p_B = 0.53$ to around 0.65. It is difficult to assess if the observed difference in estimated p_B from data sets 1 and 2 really reflects a change in allele frequencies over time or is a consequence of phenotypes recorded at different ages (6 and 5 weeks, respectively). Nonetheless, Figure 4 shows that, within a time period (data set 2), the estimated frequency of the allele that increases SaO increases slightly over time, which is consistent with the between-data-set trend.

For data set 1, the predicted proportion of heterozygous individuals would be around 50%. Because the gene is dominant, around 25% of the population would show low SaO values. Despite this, this locus alone would explain 48% of the total variance and around 87% of the genetic variance in the 'base population'. For data set 2, the predicted proportion of heterozygous individuals would be around 45% and only around 12% of the population would show low SaO values. The proportion of the total variance observed in the data set 2 'base population' explained by this putative locus has decreased to 33% and that of the genetic variance to 80%. From data set 1 to data set 2, the estimate of the major locus variance has more than halved. Changes in the major locus allele frequency

alone account for 71% of this difference compared to 46% accounted for by changes in estimated effects alone. The polygenic variance estimated from data set 2 was roughly two-thirds of the estimate obtained from data set 1. This, together with an increase in the frequency of the allele that increases SaO, is consistent with the fact that increasing SaO has been one of the breeding goals in the population studied. As a result of decreases in major gene and polygenic variances, the total and the major locus heritabilities have decreased because the residual variance has remained approximately constant. Note that the frequencies of birds with low SaO values in the two data sets do not necessarily provide a direct prediction of the expected frequency of ascites because the correlation between ascites and SaO in this population is unknown. In fact we might predict that a low SaO value predisposes a bird to ascites, but the actual development of ascites is also dependent on other environmental factors. For example, Julian and Mirsalimi (1992) showed that there was a significant mean difference in SaO between ascitic and non-ascitic birds from a heavy meat-type chicken, but not all birds with low SaO developed ascites during their trial.

Selection experiments carried out in other broiler populations to study ascites susceptibility tend to suggest that this trait is influenced by a single biallelic major locus that would act in a recessive fashion (see for example, Druyan *et al.* (2001, 2002), Wideman and French (1999, 2000)) and this would support our findings.

The mean skewness coefficient of our data sets (after adjustment for fixed effects) was around -0.77 . In an outbred situation, where one expects only a proportion of families to be segregating at a putative major locus, the trait distribution within full and/or half sib families would depend on the sire and dam major genotypes, and families that do not segregate at the major locus would only show 'background skewness' (skewness not caused by the major locus segregation). Figure 5 shows the distribution of adjusted SaO phenotypes within two sire families with over 100 offspring each. Sire 1 was assigned a *Bb* genotype with a probability greater than 0.99 in the segregation analysis whereas sire 2 was assigned a *BB* genotype with a probability greater than 0.98 and a *bb* genotype with a probability smaller than 0.02. It can be seen that dispersion within sire family 1 is larger than dispersion within sire family 2, which is consistent with the predicted genotype status of the two sires.

We observed that, for the analyses carried out, the total additive variance estimated using a mixed inheritance model was greater than the additive variance estimated using a polygenic

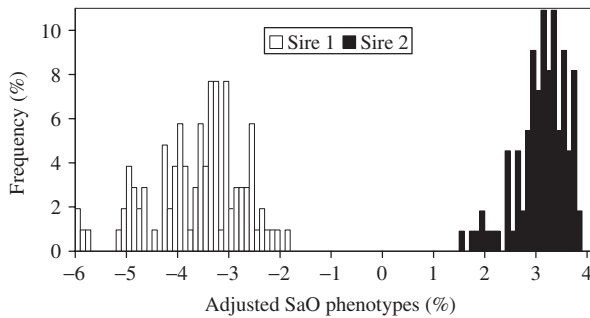


Figure 5. Distribution of adjusted SaO phenotypes within two sire families with over 100 offspring each. The genotypes of sire 1 and sire 2 were estimated to be heterozygous Bb and homozygous BB, with probabilities higher than 0.99 and 0.98, respectively.

model. We carried out a simulation study, presented in the annex, that supports the hypothesis that changes in allele frequencies caused by selection are the source of the observed discrepancies.

By regressing Weight and Flesh phenotypes on functions of major locus genotype probabilities we obtained estimates of the effects of the putative locus on these traits. The locus that has an effect on SaO seems to act in an overdominant fashion for weight and fleshing score. This would be consistent with the estimate of zero for the genetic correlation between these traits and oxygen saturation from the analysis done assuming a purely polygenic model with additive genetic effects and would also explain the intermediate frequency of the high SaO allele estimated from data set 1, which increases after selection to increase oxygen saturation. In an experiment involving the hypobaric exposure of birds, Pavlidis *et al.* (2003) observed significant heterosis for body weight at 14, 18 and 42 d in the reciprocal crosses of an ascites-resistant and an ascites-susceptible line, but observed no differences in body weight between the ascites-resistant and ascites-susceptible lines. These observations fit well with our results. Estimates of the putative gene effects for SaO obtained from this analysis were approximately twice the ones obtained from the segregation analysis. Genotype probabilities were estimated from segregation analysis and only birds with phenotypes from the tails of the SaO trait distribution (and/or strong family information) are likely to have extreme (close to 0 or 1) estimates of genotype probabilities. This would cause these individuals to have a high influence in the regression and hence could lead to an over-estimation of the locus effect for SaO. The extent to which this would affect estimates of

effects for Weight and Flesh would be a function of the true genetic correlation amongst these traits and SaO.

Conclusions and further research

Our study indicates that a QTL or gene with large effect on SaO is segregating in the population studied. It must be borne in mind that, although segregation analysis is the most powerful marker-free method for major gene or QTL detection, it is sensitive to deviations from normality, and the distribution of the data analysed was skewed. Nevertheless, the majority of the different analyses performed here are consistent with the presence of a major gene. Only the result from the analysis of transformed data (presented in the annex) provides a cautionary note, but previous studies suggest this may be expected even in the presence of a genuine major gene. Accepting the presence of a major gene, the mode of action of the putative locus on SaO and on weight and fleshing score, the fixation of the favourable allele (the one that increases SaO) by means of traditional selection would be a difficult task. Under selection only for growth and carcass characteristics, the low SaO allele would be maintained in the population by the advantage of the heterozygous bird. The combined effects of selection to increase both SaO and weight and fleshing score will reduce the fitness of the low SaO homozygote, but will still result in the heterozygote being the favoured genotype and hence the low SaO allele will be retained in the population. Nonetheless, elimination of carriers of the allele that decreases SaO is of interest because it would lead to greatly improved broiler health, would reduce the broiler industry's ascites-related economic losses and remove the need for continual SaO testing. However, given the estimated effects of the locus, this would be most effectively achieved using genetic markers to identify and select against the low SaO allele. A QTL mapping study in a suitable population is a necessary further step that would confirm or refute our findings and identify potential markers to manipulate the low SaO allele.

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Annex

METHODS

Segregation analysis

The mixed model equation that describes the model fitted to the data is:

$$y = Xb + Zu + ZWm + e \quad (1)$$

where y is the vector of phenotypic observations, b is the vector of fixed non-genetic effects and X is the design matrix relating fixed non-genetic effects to observations. Z is the incidence matrix for random polygenic effects ($u \sim N(0, A\sigma_u^2)$ – where A is the numerator relationship matrix and σ_u^2 is the polygenic variance) – and single locus effects. W is a three column matrix that contains information

on the genotype of each individual and \mathbf{m} is the vector of major-genotype means ($\mathbf{m}' = [-a, d, a]$), hence \mathbf{Wm} is the vector of random effects at the single locus. \mathbf{e} ($\sim N(0, \mathbf{I}\sigma_e^2)$) is a vector of random errors.

Janss *et al.* (1995) proposed an efficient scheme using the Gibbs sampler for the study of mixed inheritance models in animal populations. In our analyses, carried out with software developed at Roslin Institute by Ricardo Pong-Wong (Walling *et al.*, 2002), we used the sampling scheme they described (see Janss *et al.* (1995; 1997) for details) to obtain marginal posterior distributions for the major locus parameters (frequency and additive and dominance effect), population mean and polygenic and residual variances. For each iteration of the Gibbs sampler, every bird was assigned a genotype. Averaging over all retained iterations provides major locus genotype probabilities (p_{BB} , p_{Bb} and p_{bb}) for each bird in the pedigree.

The variance explained by the major locus (σ_m^2) is defined as:

$$\sigma_m^2 = 2p_B(1 - p_B)[a + d((1 - p_B) - p_B)]^2 + [2p_B(1 - p_B)d]^2 \quad (2)$$

(Falconer and Mackay, 1996) and was computed from the major locus genotypic effects and allele frequency sampled at each iteration. Likewise, we calculated the degree of dominance as d/a .

We used non-informative prior distributions, uniform on $(-\infty; +\infty)$ for fixed non-genetic effects and d , on $[0; +\infty)$ for a and on $[0; 1]$ for major allele B frequency. We used an inverse-gamma prior distribution on $(0; +\infty)$ for variances with a flat prior for $\log(\text{variance})$. This type of prior distribution for variances should cause the mean of the marginal posterior distributions to tend towards zero if the data available do not support variation of the random effects. All genotypes were initialised as Bb .

Six runs of the Gibbs sampler were carried out for the analysis of each data set, with different starting values. Differences in parameter estimates obtained from different chains may reflect problems of mixing. Individual chains were composed by 255 000 iterations that were collected after allowing for a burn-in period of 5000 iterations, keeping each 100th iteration from this point onwards. In order to assess if the burn-in period and thinning parameter we used were adequate, we studied the marginal posterior distributions of the sampled parameters obtained from each run of the Gibbs sampler. For data set 1 analyses, the

marginal posterior means, that is the parameter's *a-posteriori* expectation, the posterior standard deviations and the Monte Carlo standard deviations are reported. The Monte Carlo standard deviation of the marginal posterior mean was computed following Geyer (1992) as suggested by Sorensen *et al.* (1995) and the effective number of samples per chain, that is, the number of independent samples per chain for each parameter, was estimated. Following the results obtained when studying the behaviour of the individual chains for data set 1, no formal assessment of convergence was carried out for data set 2 analyses, but a visual inspection of individual chains was carried out for each of the analyses. After studying individual chains, the samples were pooled across chains and the mean of the pooled distribution and its standard deviation were used as summary statistics. Janss *et al.* (1995) suggested the use of the ratio of the density at $\sigma_m^2 = 0$ of the marginal posterior distribution of σ_m^2 and the density at the global mode as a criterion to test the significance of the single locus component. They inferred the presence of a single locus (0.05 significance level) if the density at the global mode was 20 times larger than that at $\sigma_m^2 = 0$. We used the same criterion.

In order to ease computation, the segregation analyses were carried out using SaO phenotypes adjusted for the fixed effects of hatch week (210 and 133 levels, respectively, for data sets 1 and 2) and age of the dam at laying (10 and 9 levels, respectively, for data sets 1 and 2). Adjusted SaO phenotypes were obtained from trivariate analyses of SaO, Weight and Flesh data, performed fitting an animal model within a Restricted Maximum Likelihood (REML) framework using ASREML (Gilmour *et al.*, 2000). These analyses assumed that all three traits are under the genetic control of an infinite number of loci with small additive effects. If a major locus was involved in the genetic control of a trait, its segregation variance would contribute to the estimated σ_u^2 with the remainder included in the estimated σ_e^2 , together with polygenic non-additive variance, since the infinitesimal model does not accommodate non-additive genetic variation (although it can be extended to do so) or changes in variance caused by changes in major locus allele frequency (Turelli and Barton, 1994). Only adjusted phenotypes from birds that originally had SaO records were used, but the pedigree included contemporary unrecorded birds. This allowed us to obtain genotype probabilities for all birds in the pedigree.

Analyses of genotype probabilities and allele frequencies

Some exploratory analyses were carried out on genotype probabilities obtained from data set 2 analyses. From each iteration of the Gibbs sampler, genotype configurations were obtained for all birds in the pedigree. Averaging over all retained iterations, probabilities of each bird being *BB*, *Bb* or *bb* could be obtained. For each bird, as many sets of genotype probabilities as chains were produced and an overall estimate of each genotype probability was obtained by averaging the results from each chain. Genotypic frequencies at a given moment in time could be obtained by averaging the frequencies of birds in the chosen period and estimates of major allele frequencies can be obtained as $p_B = p_{BB} + 0.5 p_{Bb}$ and $p_b = p_{bb} + 0.5 p_{Bb}$. As genotype probability estimates are a function of the individual's phenotypic record and information from its relatives, we divided the population on the basis of the amount of information available for each individual in birds with no record, selection candidates and sires. Most of the results presented will be for the two last categories because accuracy of estimates should be highest for sires but using estimates from selection candidates as well one can attain a compromise between accuracy of estimates and sample size.

Estimation of the putative locus effect on weight and fleshing score

In order to investigate the effect of the putative major locus on body weight and fleshing score measured at 5 weeks of age, the phenotypic values for these traits were regressed on functions of the genotype probabilities estimated from the segregation analysis of data set 2. The model used was:

$$y = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Sc} + \mathbf{Rg} + e \quad (3)$$

with elements defined as in [1] and \mathbf{c} , \mathbf{S} , \mathbf{g} and \mathbf{R} are, respectively, the vector of random maternal environmental effects for Weight and Flesh and the design matrix relating maternal environmental effects to observations ($\mathbf{c} \sim N(0, \mathbf{I}\sigma_{em}^2)$) and the vector of random maternal genetic effects for Weight and Flesh and the design matrix relating maternal environmental effects to observations ($\mathbf{g} \sim N(0, \mathbf{A}\sigma_{gm}^2)$). \mathbf{X} now includes $c_a = (p_{BB} - p_{bb})$ and $c_d = p_{Bb}$, allowing one to estimate, respectively, the additive and the dominance effect of the putative locus on the traits. A trivariate analysis was carried out within a REML framework, fitting an animal model using ASREML (Gilmour *et al.*, 2000).

RESULTS

Segregation analyses

For all runs and parameters, chains seemed to have converged to their equilibrium distributions after a 5000 iteration burn-in period and sample autocorrelation was generally low (absolute values smaller than 0.1) for lags over 100 for all parameters (results not shown). Marginal posterior distributions of all parameters were symmetric and approximated normal distributions. Table A1 shows the marginal posterior means, posterior standard deviations, Monte Carlo standard deviation of marginal posterior means and the effective number of samples per chain for all sampled parameters. The difference between marginal posterior means from the six chains ran for data set 1 was not always strictly within the Monte Carlo sampling error estimated as proposed by Geyer (1992). Nonetheless, all chains seemed to have converged to the equilibrium distribution (using different starting values) and marginal posterior means were very close. The effective number of samples was variable within chains between parameters and within parameters between chains, with values ranging from nine to more than 600 independent samples. Generally σ_u^2 and σ_e^2 showed smaller effective numbers of samples, which reflects poorer mixing for these parameters.

The visual inspection of the 6 chains did not show convergence problems.

Samples were pooled across chains and the means of the pooled distributions and their standard deviations were used as point estimates of the sampled parameters and their standard errors. As the total number of independent samples for any parameter was greater than 100, the mean and the standard deviation of the pooled posterior distribution were assumed to be good estimates of the parameter and its standard error. Table 4 shows point estimates of the parameters sampled and those derived and the standard deviation of their pooled posterior distributions. All distributions presented zero densities for parameter values equal to zero. Following Janss *et al.* (1995) we inferred that a locus with large effect on SaO was segregating in the population studied. Although the estimated d was slightly larger than a , estimated major locus effects were similar in size (the dominance deviance was estimated to be 1.12 (± 0.06)) and was indeed just different from one. We will assume in the following that a and d can be considered equal.

Analyses were repeated for data set 2, using raw phenotypes (records not adjusted for fixed effects) and sampling fixed effects (age of dam and hatch). These analyses yielded estimates of

Table A1. Descriptive statistics of chains obtained from the analysis of data set 1. Marginal posterior means (MPM), posterior standard deviations (PSD), Monte Carlo standard deviations (MCSD) of MPMs and effective number of independent samples (ENS) per chain for the major locus additive (a) and dominance (d) effect, B allele frequency (p_B), population mean (Mean) and residual (σ_e^2) and polygenic (σ_u^2) variances. A mean ENS is also shown for each sampled parameter

Parameter	Chain	a	d	p_B	Mean	σ_e^2	σ_u^2
MPM	1	7.236	8.089	0.528	-5.642	35.679	5.990
	2	7.201	8.049	0.524	-5.585	35.320	6.185
	3	7.241	8.034	0.524	-5.587	35.584	6.232
	4	7.158	8.113	0.526	-5.594	35.553	6.092
	5	7.265	8.011	0.527	-5.597	35.817	5.775
	6	7.214	8.025	0.525	-5.597	35.550	6.062
PSD	1	0.205	0.321	0.026	0.339	1.213	1.037
	2	0.192	0.315	0.026	0.338	1.029	0.939
	3	0.201	0.318	0.026	0.345	1.181	0.988
	4	0.187	0.323	0.026	0.322	1.080	0.961
	5	0.203	0.313	0.026	0.331	1.139	0.926
	6	0.194	0.321	0.026	0.327	1.142	1.040
MCSD	1	0.053	0.032	0.002	0.045	0.409	0.200
	2	0.011	0.037	0.001	0.038	0.110	0.141
	3	0.042	0.027	0.001	0.033	0.241	0.216
	4	0.013	0.046	0.001	0.035	0.162	0.259
	5	0.040	0.024	0.001	0.028	0.321	0.172
	6	0.016	0.023	0.001	0.026	0.213	0.172
ENS	1	15	102	138	56	9	27
	2	310	72	421	81	87	44
	3	23	143	410	109	24	21
	4	205	49	607	83	45	14
	5	25	174	516	137	13	29
	6	143	194	583	163	29	37
Mean ENS	-	120	122	446	105	34	29

the parameters presented in Table 4 that were similar to the ones obtained from adjusted phenotypes (results not shown), showing that, in this case, pre-adjustment of phenotypes did not have a significant effect on parameter estimates.

DISCUSSION

On skewness

When a major locus is segregating, the population distribution of phenotypes can be skewed. MacLean *et al.* (1975) showed that skewness of the phenotypic distributions, when not caused by segregation of loci with large effect, could lead to detection of a spurious major locus. MacLean *et al.* (1976) suggested applying a transformation to the phenotypic data to remove skewness prior to analysis, but showed that this could considerably reduce the power to detect major genes when present, as well as posing problems for the interpretation of the results (Demenais *et al.*, 1986). Using data set 2, we applied a transformation to SaO data, in order to obtain a trait distribution closer to a Normal. The transformation applied was $\text{Ln}(100 - \text{SaO})$, and the transformed trait distribution had a skewness coefficient of -0.29 and a kurtosis coefficient of -0.17 .

The heritability of $\text{Ln}(100 - \text{SaO})$ was 0.14 , which is almost identical to that obtained for untransformed data. The outcome of the analysis of transformed data was that a dominant locus with large effect on $\text{Ln}(100 - \text{SaO})$ ($a=d=0.58 \sigma_p$) was segregating in this line, but neither the frequency nor the mode of action of this putative locus were in agreement with the results obtained from untransformed data. Indeed, an allele that increases $\text{Ln}(100 - \text{SaO})$ would decrease SaO, so the estimated $p_B=0.69$ from this analysis needs to be compared to $(1-0.65)=0.35$. In the same way, if the locus that increases $\text{Ln}(100 - \text{SaO})$ were dominant, the proportion of birds showing low SaO values would be around 81% compared with the predicted 12% from the untransformed data analysis. Moreover, the correlation between the probability of a bird being heterozygote estimated from transformed and untransformed data was -0.17 ($P<0.001$) for birds with SaO data and -0.11 ($P=0.03$) for sires with SaO data. The fact that the estimates of p_{Bb} are not similar from transformed and untransformed data suggests that these analyses are describing different phenomena. Selection experiments carried out in other broiler populations to study ascites susceptibility tend to suggest that this trait is influenced by a single biallelic

Table A2. Simulated parameters (in brackets at table heading) and estimates (with standard deviations) obtained assuming either a purely polygenic model (POLF) or a mixed inheritance model (MIX). Estimates were obtained by averaging estimates from 125 replicated populations. *a* and *d* are the major locus additive and dominance effect and *p_B* is the B allele frequency. Residual (σ_e^2) and polygenic (σ_u^2) variances are shown, as are major locus additive, dominance and total variances (σ_{am}^2 , σ_{dm}^2 and σ_m^2), total phenotypic variance (σ_p^2), total additive variance (σ_a^2) and variance ratios (*h_T*, *h* and *h_a*). Results are presented for populations under phenotypic (PHE) or random (RAN) selection.

Selection	Model	<i>a</i> ¹ (6.46)	<i>d</i> (6.60)	<i>p_B</i> (0.65)	σ_e^2 (32.67)	σ_u^2 (3.93)	σ_{am}^2 (9.13)	σ_{dm}^2 (9.02)	σ_m^2 (18.15)	σ_p^2 (54.75)	σ_a^2 (13.06)	<i>h_T</i> (0.40)	<i>h</i> (0.11)	<i>h_a</i> (0.24)
PHE	POLF	NA	NA	NA	39.31 (1.18)	6.68 (0.48)	NA	NA	NA	45.99 (1.18)	6.68 (0.48)	NA	0.17 (0.01)	NA
	MIX	6.77 (0.49)	5.99 (0.58)	0.66 (0.03)	32.02 (1.14)	5.01 (0.68)	10.32 (2.24)	7.11 (1.20)	17.43 (2.20)	54.46 (2.16)	15.33 (1.97)	0.41 (0.03)	0.14 (0.02)	0.28 (0.03)
RAN	POLF	NA	NA	NA	39.78 (1.41)	15.17 (2.25)	NA	NA	NA	54.95 (2.79)	15.17 (2.25)	NA	0.38 (0.06)	NA
	MIX	6.49 (0.35)	6.42 (1.27)	0.65 (0.03)	32.73 (1.48)	3.76 (1.40)	9.53 (3.35)	8.77 (1.67)	18.30 (2.82)	54.79 (3.08)	13.29 (3.45)	0.40 (0.04)	0.10 (0.04)	0.24 (0.04)

$$\sigma_{dm}^2 = 2 p_B(1 - p_B) [a + d((1 - p_B) - p_B)]^2$$

$$\sigma_{dm}^2 = [2p_B(1 - p_B)d]^2$$

$$\sigma_m^2 = \sigma_{am}^2 + \sigma_{dm}^2$$

$$\sigma_p^2 = \sigma_m^2 + \sigma_u^2 + \sigma_e^2$$

$$\sigma_a^2 = \sigma_{am}^2 + \sigma_u^2$$

$$h_T = (\sigma_m^2 + \sigma_u^2) / \sigma_p^2$$

$$h = \sigma_u^2 / (\sigma_u^2 + \sigma_e^2)$$

$$h_a = (\sigma_{am}^2 + \sigma_u^2) / \sigma_p^2$$

major locus that would act in a recessive fashion (see for example, Druyan *et al.* (2001, 2002), Wideman and French (1999, 2000)). This would support the mode of action suggested for SaO by the analysis of untransformed data.

On variance components estimates (simulation)

We observed that, for the analyses carried out, the total additive variance estimated using a mixed inheritance model was greater than the additive variance estimated using a polygenic model. In order to assess whether this would be expected in the presence of a segregating major gene in a population under selection a simulation study was performed. A total of 125 five-generation pedigrees (base population and 4 generations of random or phenotypic selection) were simulated with a structure chosen to resemble the (real) pedigree analysed. In each generation, 40 males were mated to 8 (different) females that produced three male and three female offspring each (that is, population size was maintained constant). In the case of phenotypic selection, the 40 males and 320 females with highest phenotypes were selected to produce the next generation. The simulated pedigrees consisted of 9600 individuals. Phenotypes were assumed to be under the control of a gene with large effect and a large number of polygenes with small additive effect, as well as partially determined by the environment. The parameters used for the

simulation were the ones presented in Table 4 for data set 2. Data from all animals or from males alone (to mimic our data structure) were analysed assuming an infinitesimal model, within a REML framework, using ASREML (Gilmour *et al.*, 2000). In a second stage, the pedigrees were analysed assuming a mixed inheritance model (as described in the Materials and Methods section) and only data from males were used. Table A2 shows the means and standard deviations of the parameters estimated. In brief, the simulation study showed that, in a population in which both a major gene and polygenic variation are actually present and which is under phenotypic selection, the estimate of the total additive variance is severely biased downward if a purely polygenic model is assumed. Estimates from REML of additive variances obtained by simulation are in close agreement with results obtained when analysing real data. Assuming a change in major allele frequencies like the one shown in Figure 4 for selection candidates, we estimated that total additive variance would be underestimated by around 22% when assuming a purely polygenic model. Results from simulation show that this underestimation is around 31%. This, together with the fact that total additive variance seems to be well estimated (only slightly overestimated) under random selection, both assuming purely polygenic or mixed inheritance, would support the hypothesis that changes in allele frequencies caused by selection are the source of the observed discrepancies.