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Genomic prediction of bone strength in laying hens using different sources of information

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ABSTRACT

Bone damage in laying hens remains a significant welfare concern in the egg industry. Breeding companies rely on selective cross-breeding of purebred birds to produce commercial hybrids, which farmers raise for table-egg production. Genomic prediction is a potential tool to improve bone quality in laying hens. Because commercial layers are crossbred and kept in different environments than pure lines, the question arises whether to use within-line purebred selection or whether to use crossbred data. While selection based on pure line data is common, achieving optimal bone strength in hybrids may require incorporating hybrid data to account for heterosis and housing-specific effects. This study aims to evaluate how combining pure line and hybrid data could affect the accuracy of breeding values for bone strength. Genotypes and phenotypes were available from two types of white hybrids (Bovans White and Lohmann Selected Leghorn Classic) housed in two housing systems (furnished cages and floor housing). This resulted in four hybrid-housing combinations (n ~ 220 for each). Tibia strength and genotypes for pure breeding lines of White Leghorn (**WL**, n = 947) and Rhode Island Red (**RIR**, n = 924) were also included. Each of the hybrid-housing combinations and pure lines was fitted separately into (1) single-trait Genomic Best Linear Unbiased Prediction (**GBLUP**), then simultaneously via multitrait GBLUP, (2) within hybrids across housing, (3) across hybrids within housing, (4) across hybrids and housing, (5) the latter in combination with WL and/or RIR data. Including hybrid data slightly increased the accuracy of the genomic estimated breeding value (**GEBV**) of other hybrids, but not that of pure lines. Pure line data increased the GEBV accuracy of hybrids over and above that of combining hybrid information. Combining data from two pure lines improved the GEBV accuracy of both. In comparison to the combination of data across lines and/or houses, combining tibia strength and BW within-lines increased tibia strength GEBV accuracy. The maximum GEBV accuracy obtained for tibia strength ranged from 0.42 to 0.65 for hybrids and from 0.63 to 0.78 for pure lines. Further study is required to test whether modelling the interactions of genotype by environment could help to breed hybrids for specific housing systems.

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Implications

Bone damage in laying hens is a major welfare challenge, but the moderate heritability of bone strength suggests selective breeding for stronger bones as a key option. Selective breeding of purebreds creates hybrids, but the housing of purebreds and hybrids may differ. Integrating bone data of purebreds and hybrids could optimise selection for hybrids' housing. This study evaluates how combining data from purebreds and hybrids would affect the breeding value accuracy of bone strength. Hybrid data slightly improved breeding value accuracy for other hybrids but not for purebreds. Purebred data increased breeding value accuracy for hybrids and other purebreds.

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Introduction

Bone damage in laying hens remains a major welfare challenge in the egg industry, with high prevalence among commercial layers (Thøfner et al., 2021; Grafl et al., 2017; Heerkens et al., 2016; Riber and Hinrichsen, 2016; Käppeli et al., 2011; Wilkins et al., 2011; Gregory and Wilkins, 1989). The problem is complicated because bone damage has different components: genetic (Sallam et al., 2023; Dunn et al., 2021; Bishop et al., 2000), environmental, including housing (Fleming et al., 2006), and potentially genotype-by-environment interaction (Johnsson et al., 2022). Commercial layers, referred to as hybrids or crossbred, are produced from a four-way cross of pure lines and are kept in different environments than pure lines. The differing genetic compositions and environments between pure lines and hybrids, raise questions about whether to utilise only purebred data for breeding purposes or incorporate also data from the crossbred layers.

The most obvious breeding strategy would be to record bone strength in pure lines and perform within-line (genomic) selection. However, the target is to enhance bone quality in hybrids. If the genetic differences and genotype-by-environment effects are substantial enough, selection based on hybrid data may be necessary, and could be achieved with the aid of genomics. Such selection could proceed according to four steps: (1) estimate genomic breeding values of hybrids' bone strength, then (2) use the marker effects from such a model for selection within the pure lines, (3) quantify the genetic gain for bone strength in the hybrids, i.e., grand-offspring of the selected individuals. While the goal is to select within pure lines for the commercial hybrid performance, the current study focuses on the first step.

Modern commercial hybrid layers are the product of crosses of different genetic lines. White Leghorn (**WL**) and Rhode Island Red (**RIR**) are two important breeds used for the production of white and brown commercial hybrids. Both WL and RIR are selected for egg production, but they are genetically distinct. In WL, earlier maturity (or age at first egg) displayed unfavourable genetic correlation with BW and tibia breaking strength. In RIR, on the other hand, bigger egg mass displayed unfavourable genetic correlation with age at first egg and tibia breaking strength (Dunn et al., 2021). These patterns (regarding correlations between egg, BW, and bone) may be sustained, mitigated or even exacerbated in the outcomes of all possible crosses (hybrids) of WL and RIR, a process that is governed by heterosis (Isa et al., 2020).

Housing is an important environmental component of bone damage (Fleming et al., 2006). Conventional battery cages, furnished cages, and non-cage systems (such as floor or aviary setups) are commonly used. In conventional cages with limited movements, bones tend to be weaker than bones from housing offering more opportunity for movements (Fleming et al., 2006), but the tibia bone may develop a little more strength if birds tend to stand more (Silversides et al., 2012). In conventional cages, keel fractures are less common during the laying period (Sandilands, 2011; Sherwin et al., 2010), but more frequent during depopulation, compared to both furnished cages and non-cage housing (Sherwin et al., 2010). Furnished cage and non-cage housing promote the strength of bones (tibia, humerus and keel) due to increased movement opportunities and access to items such as perches or multi-tiers of the system, compared to the conventional cages (Leyendecker et al., 2005; Fleming et al., 2004). However, non-cage housing poses a higher risk of collision accidents and severe bone damage, particularly to the keel bone (Petrik et al., 2015; Sandilands, 2011). Keel fractures are more frequent in non-cage housing than in furnished cages and conventional cages. (Thøfner et al., 2021). Despite the rise in non-cage and furnished cage egg markets, conventional cages remain predominant. Developing selection criteria for hybrids that foster strong bones with less damage for specific housing systems is a key challenge.

As housing environments of hybrids are varied, and not necessarily identical to that of breeding pure lines, there is a potential for genotype-by-environment interactions. The selected lines of high bone strength that were described by Bishop et al. (Bishop et al., 2000) resulted in reduced keel damage; however, that reduction was less in aviary than in cages (Fleming et al., 2006). Johnsson et al. (Johnsson et al., 2022) showed that GWAS significant markers on tibia strength are different in caged versus non-caged hybrids. Also, in wing bones, the improvement in the radius when comparing battery cages to a non-cage housing was not to the same extent in brown as in white hybrids (Silversides et al., 2012), suggesting a different pattern of genotype by environment interaction in white and brown hybrids. This suggests that data from several genetically and environmentally distinct sources are needed to select laying hens for optimal bone quality.

Laying hens have a relatively low genetic diversity. The populations of commercial layers (and broilers) are less varied than non-commercial ones (Zhang et al., 2020; Muir et al., 2008). Muir et al. (Muir et al., 2008) suggested three reasons for low diversity in commercial populations: (1) only a few breeding organisations supply the majority of commercial layers, (2) a limited number of breeds are utilised to produce the commercial layers, (3) commercial layers are the end-product of within-breeding company intensive selections followed by a pyramid expansion, analogous to a bottleneck event. The intensive selection suggests extensive linkage disequilibrium (**LD**) and lower effective population size, the advantage of this is a possibility of genomic prediction with relatively small reference populations (Hayes et al., 2009). Moreover, the moderate to strong heritability of tibia strength (Johnsson et al., 2022) would facilitate genomic prediction. The objective of the present study is to evaluate how combining information across populations (pure lines and hybrids) would affect the breeding value accuracy of tibia strength.

Material and methods

Animals, management and housing

The hybrids in the present study are the same as in (Johnsson et al., 2022; Wall et al., 2022), a cohort of Bovans White (n = 437) and Lohmann Selected Leghorn Classic (LSL, n = 436). Bovans White (n = 220) and LSL (n = 218) destined for furnished cages were contained in one of the tiers of an aviary to resemble rearing in a conventional rearing cage until 15 weeks of age. Bovans White (n = 217) and LSL (n = 218) destined for non-cage housing were reared in an aviary system with full access to all tiers. At 15 weeks of age, the pullets were transferred from the rearing facility to the poultry experimental facility at the Swedish Livestock Research Centre Lövsta (Uppsala, Sweden) and subsequently housed either in furnished 8-hen cages or in a one-tier floor housing system with 102 laying hens per group. Full details of housing and management are described in (Wall et al., 2022). The purebred lines, WL and RIR in the present study are the same as in (Dunn et al., 2021). Cohorts of 947 WL and 924 RIR hens from a pure breeding line of Lohmann white and brown commercial layers (Lohmann Breeders GmbH, Germany). The WL and RIR hens from eight hatches were assigned to two houses with cages equipped with perches (two birds per cage).

Bone strength phenotypes

The bone phenotypes were available from previous studies (Johnsson et al., 2022; Dunn et al., 2021), where hens of WL, RIR

and hybrids were euthanised at 100, 68 and 100 weeks of age, respectively. Then, BWs were recorded, and the tibia bones were collected for further detailed post-mortem bone measurements. In the current study, we included only the measurements of tibia breaking strength measured by the same method in all cohorts through a three-point bending test using a material testing machine (JJ Lloyd LRX50, Sussex, UK) as described by Fleming et al. (Fleming et al., 1994).

Genotyping

A total of 2 744 hens: 437 Bovans White, 436 LSL, 947 WL and 924 RIR were genotyped for 57 636 single nucleotide polymorphisms (SNPs) using the Illumina Infinium assay. The genotyping was performed by the SNP&SEQ Technology Platform (Uppsala University, Sweden). Sequences were aligned flanking the markers against the GRCg6a chicken reference genome (Warren et al., 2016) to determine the physical positions of the SNPs. A total of 17 358 SNPs were removed because of being monomorphic or having a low call rate (< 0.90) or minor allele frequency (< 0.05). After all quality control checks, a total of 40 278 SNP markers were retained for further analysis.

Genomic prediction

We analysed the tibia strength of two white hybrids (Bovans and LSL) kept in two housing systems (cage and non-cage), and the pure breeding lines WL and RIR. The tibia strength of pure line and hybrid-housing combinations were treated as different but correlated traits, and designated with hybrid and housing codes (e.g., Bovans-cage stands for the white hybrid Bovans housed in cages). This resulted in six data classes of tibia strength: four classes (hybrid-housing combinations) from the hybrids' data, and two classes from the pure lines WL and RIR data. The variability of phenotypic data in hybrids and pure lines are summarised in Table 1.

To obtain genomic predictions for each hybrid-housing combination of tibia strength, we investigated the following scenarios:

1. predictions based on a single data class, i.e., each hybrid-housing combination of tibia strength is analysed separately via single-trait Genomic Best Linear Unbiased Prediction (**GBLUP**).
2. predictions based on combining data classes simultaneously within hybrids across housings, e.g., LSL-cage and LSL-non-cage, via two-trait GBLUP.
3. predictions based on combining data classes simultaneously across hybrids within housings via two-trait GBLUP. Combining data across hybrids from different breeding organisations (e.g., Bovans-cage and LSL-cage) is unlikely to happen in practice; however, it represents the extreme situation of combining data of different hybrids within the same breeding organisation.
4. predictions based on combining data classes simultaneously across hybrids and housings via four-trait GBLUP.
5. predictions based on combining data as in scenario 4 plus WL, or RIR data simultaneously via five-trait GBLUP.
6. predictions based on combining data as in scenario 4 plus WL and RIR data simultaneously via six-trait GBLUP.

Scenarios 3 and 4 were designed to investigate how combining data within (or across) hybrids and housings is relevant for predicting the tibia strength of each hybrid-housing combination. Scenarios 5–6 were designed to investigate how combining the relatively large data of the breeding pure lines WL and/or RIR with these hybrid-housing combinations is relevant for predicting the tibia strength of each hybrid-housing combination.

Single-trait and multitrait genomic best linear unbiased prediction

We used a conventional genomic animal model: y = X b + Z u + e, where y is a vector of standardised trait measurement, X is a design matrix that relates measurements y to b vector of the confounding fixed effects, including feed (in hybrids), hatch (in WL and RIR), and the covariate BW (in hybrids, WL and RIR). Z is a design matrix that relates the measurements y to u vector of the random animal (or hen) effects. In single trait GBLUP, the random animal effects are obtained based on contributions from the genomic relationship matrix G, and λ variance component ratio ($\lambda = \sigma^2_e / \sigma^2_u$), where σ^2_u is the additive genetic variance and σ^2_e is the residual variance. In multitrait GBLUP, the animal effects have additional contributions through the genetic covariance structure with other traits, but the residual covariance between traits is assumed to be zero.

The mixed model equations for single trait GBLUP:

$$\begin{bmatrix} \hat{b} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + \lambda G^{-1} \end{bmatrix} -1 \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

The mixed model equations for e.g., two-trait GBLUP:

$$\begin{bmatrix} \hat{b}_1 \\ \hat{b}_2 \\ \hat{u}_1 \\ \hat{u}_2 \end{bmatrix} = \begin{bmatrix} X'_1X_1 & 0 & X'_1Z_1 & 0 \\ 0 & X'_2X_2 & 0 & X'_2Z_2 \\ Z'_1X_1 & 0 & Z'_1Z_1 + G^{-1}\lambda_1 & G^{-1}\sigma^2_{e1}/\sigma_{u12} \\ 0 & Z'_2X_2 & G^{-1}\sigma^2_{e2}/\sigma_{u12} & Z'_2Z_2 + G^{-1}\lambda_2 \end{bmatrix} -1 \begin{bmatrix} X'_1y_1 \\ X'_2y_2 \\ Z'_1y_1 \\ Z'_2y_2 \end{bmatrix}$$

Where subscripts refer to trait 1 and trait 2, and σ_{u12} is the additive genetic covariance between trait one and trait two.

Estimation of genomic best linear unbiased prediction parameters

The variances and covariances (and associated errors) were estimated separately for each scenario, using the genomic restricted maximum likelihood (GREML) as implemented in AIREMLF90 package (Misztal et al., 2022). The G genomic relationship matrix was constructed as described by VanRaden (VanRaden, 2008):

Table 1
Variability in phenotypic data among hybrids and pure lines of laying hens.

	BW (Kg)		Tibia strength (Newton)			n
Data classes	Mean[1]	CV%	Mean[1]	CV%	correlation with BW	
Bovans-cage	1.93[a]	10.01	147.5[d]	22.11	0.33	220
LSL-cage	1.87[bc]	11.2	152.79[d]	22.78	0.31	218
Bovans-non-cage	1.91[ab]	10.41	212.83[bc]	27.24	0.28	217
LSL-non-cage	1.86[c]	9.73	208.52[c]	22.49	0.33	218
WL	1.71[d]	7.3	225.14[ab]	32.78	0.13	947
RIR	1.91[a]	9.7	230.35[a]	24.19	0.25	924

Abbreviations: Bovans = White Bovans hybrid; LSL = Lohmann Selected Leghorn Classic hybrid; WL = White Leghorn; RIR = Rhode Island Red.
[1] Comparing means was based on Tukey's all-pairwise comparisons with *P*-value < 0.05, groups with different (same) letters have statistically different (not different) means.

$G = \frac{W W'}{\sum 2p_i(1-p_i)}$ where W is a matrix of n genotyped individuals × m SNP markers and contains marker alleles coded as 0, 1, and 2 for common allele homozygous, heterozygous, and rare allele homozygous, respectively. Each column of W represents SNP for one genetic locus and was centred by subtracting the *p* rare allele frequency from its elements. Elements of G are then scaled by the denominator $\sum 2p_i(1-p_i)$ which represents the variability of the genotypic data. Centring and scaling of G are based on allele frequencies of genotypes available in each scenario. The heritability estimates from multitrait scenarios were adjusted to reflect the corresponding population not the pooled populations as described in (Wientjes et al., 2017). The mixed model GBLUP equations were solved by iteration on data using preconditioned conjugate gradient iteration as implemented in BLUPF90 package (Misztal et al., 2022).

Accuracy and bias of genomic predictions

The genomic estimated breeding values (**GEBV**) for tibia strength, from each scenario, were evaluated in terms of accuracy and bias. A five-fold cross–validation was applied on each scenario. We re-ran each scenario 5 times with reduced data (validation runs), where phenotypes but not genotypes were set to be missing in 20% of individuals in each of the hybrid-housing combinations. The individuals with missing phenotypes (validation individuals) were randomly selected without replacement, resulting in five different sets corresponding to five validation runs. The validation individuals did not change across scenarios, so the results of validations (accuracy and bias) are comparable across scenarios. For validation individuals, the correlations between GEBV from the validation run and the tibia strength phenotype adjusted for BW were calculated. These values, referred to as GEBV accuracies, were then averaged over the five validation runs and divided by the square root of the corresponding heritability. The SD of accuracies from the five validation runs was weighted by square root of five, as the standard error of GEBV accuracy.

For validation individuals, the phenotypes adjusted for BW were regressed on the genomic prediction from the corresponding validation run. The regression intercept (b_0) and coefficient (b_1) were averaged over the five validation runs and interpreted as the prediction bias (mean and dispersion bias, respectively). The ideal unbiased prediction is supposed to have a value of zero for b_0 and 1 for b_1. The validation procedures were designed to accommodate different magnitudes of genetic correlation between pure lines and hybrids, considering the data availability in the current study. In cases of weak genetic correlation between pure lines and hybrids, hybrid data are crucial. In the ideal case, one would validate the GEBVs of pure line birds with data from their hybrid offspring. However, data on the offspring of pure lines were not available. Instead, we validated the GEBVs of hybrids for hybrid traits, and the GEBVs of pure line birds against pure line phenotype. In cases of strong genetic correlation between pure lines and hybrids, pure line data are more informative about hybrid phenotype, so that the value of hybrid data declines. This applies when pure lines and hybrids are housed similarly, and there is no heterosis for bone traits.

Patterns of linkage disequilibrium across populations and principal component analysis of genotypes

Genotypes from classes of tibia strength were assumed to represent separate populations, resulting in six populations: four hybrids (Bovans-cage, LSL-cage, Bovans-non-cage, LSL-non-cage) plus WL and RIR pure line breeding populations. The genotypes were split by chromosomes, to calculate LD within each chromosome and for SNP pairwise with distances less than 250 kilobase pair. The resulting values of pairwise LD for each chromosome were joined from the six populations into one file, to calculate correlations of pairwise LD (per chromosome) across the six populations. The values of pairwise LD across the six populations were weighted (multiplied by the number of SNPs pairwise in the respective chromosome, then divided by the number of SNPs pairwise in all chromosomes) and then summed over all chromosomes. We used the linkage disequilibrium statistics: $r = P_{AB} - P_A P_B / (P_A P_B P_a P_b)^{0.5}$ where *P* is the frequency, *A*/*a* is the first/second allele at certain locus and *B*/*b* is the first/second allele at another locus. P_{AB} is the frequency of genotypes (haplotype) that have alleles *A* and *B* in two different loci, and estimated by the maximum likelihood algorithms, as implemented in PLINK software (v1.90b6.24). Principal component analysis was used to cluster individuals with similar genotypes. Genotypes of hybrids (Bovans and LSL) and pure lines (RIR and WL) were all combined and fitted by the "pca" function in PLINK software. The resulted files of eigenvalues and eigenvectors were then visualised using R package "ggplot2".

Results

On the phenotypic level (Table 1), tibia strength tends to be higher in pure lines (RIR and WL) than in hybrids. The tibia strength of hybrids was higher in non-cage than in cage housing. While WL is lighter than RIR and hybrids, WL tibia strength was similar to RIR and higher than most of hybrids. The phenotypic correlation between tibia strength and BW ranged from 0.13 to 0.33. The first and second principal components accounted for ~57% of the variability in the genotype data of all pure lines and hybrids (Fig. 1). Bovans and LSL hybrids were clustered together, while the pure lines WL and RIR clustered separately. The first principal component suggested that hybrids were closer to WL than to RIR pure lines. However, on the second principal component, hybrids were closer to RIR than to WL pure lines. The patterns of LD in the same hybrids but housed in different systems (cage or non-cage) were similar, with a high correlation of 0.84–0.87 (Table 2). In different hybrids (Bovans or LSL) that were housed on the same or different housing systems, the LD patterns were similar, with correlation coefficients of ~0.55, and increased to ~0.60 when the LD analysis based on hybrids genotypes only (details not shown). Patterns of LD in the hybrids were ~0.30 correlated (similar) to the WL's LD patterns and only 0.07 to the RIR's LD patterns.

Genetic parameter estimates

The estimated heritability and genetic correlations tended to be less noisy in scenarios with larger data size (Table 3). The heritability estimates from multitrait scenarios were adjusted to reflect the corresponding population not the pooled populations as described in (Wientjes et al., 2017). The estimates of tibia strength heritability ranged from 0.11 to 0.66 for pure lines and hybrids. The estimates of genetic correlation between cage and non-cage environments were consistently moderate to high positive. The estimates of tibia strength genetic correlation between Bovans and LSL were high within the non-cage environment, but low in the cage environment. Estimates of genetic correlations of WL were stronger with LSL-cage (0.64) and Bovans-non-cage (−0.23) than with Bovans-cage and LSL-non-cage. Estimates of genetic correlations of RIR were stronger with LSL-housing combinations (0.65–0.85) than with Bovans-housing combinations (0.10–0.75). However, generally, estimates of correlations were uncertain.

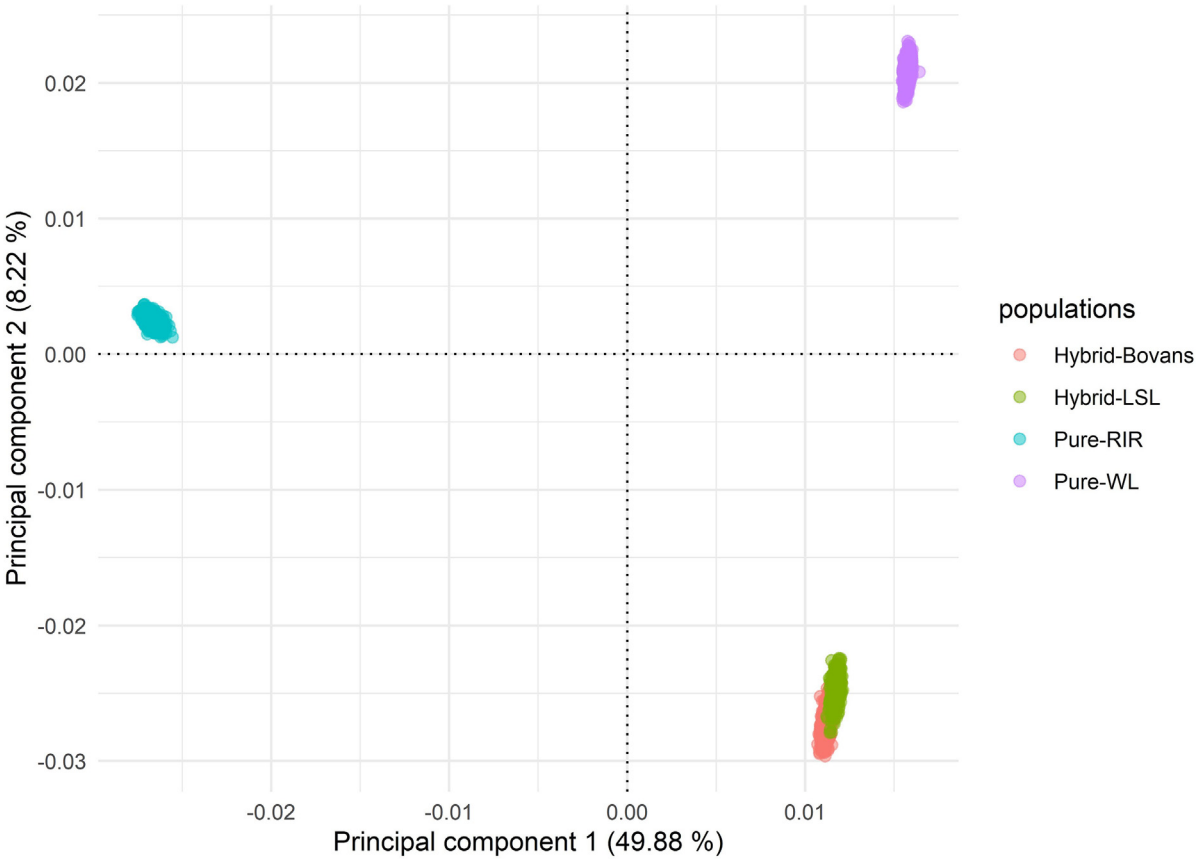

Fig. 1. Principal components of genotypes in hybrids and pure lines of laying hens. Scatterplot of the first and second principal components, showing the variance explained. Abbreviations: Bovans = White Bovans hybrid; LSL = Lohmann Selected Leghorn Classic hybrid; WL = White Leghorn; RIR = Rhode Island Red.

Table 2
Correlations[1] between patterns of linkage disequilibrium[2] across populations of laying hens.

Populations	Bovans-cage	LSL- cage	Bovans-non-cage	LSL- non-cage	WL	RIR
Bovans-cage						
LSL-cage	0.55					
Bovans-non-cage	0.84	0.54				
LSL-non-cage	0.55	0.87	0.54			
WL	0.25	0.30	0.26	0.30		
RIR	0.07	0.07	0.07	0.07	0.09	

Abbreviations: Bovans = White Bovans hybrid; LSL = Lohmann Selected Leghorn Classic hybrid; WL = White Leghorn; RIR = Rhode Island Red.
[1] number of observations used to calculate correlations is 52 790.
[2] measured by statistic r for single nucleotide polymorphism pairwise with distances less than 250 kb.

Accuracy and bias of genomic predictions for tibia strength

Combining data across hybrids slightly increased the accuracy of hybrid GEBV. Compared to the single-trait accuracy, combining data across hybrids slightly increased GEBV accuracy (1–8 units) for LSL-cage, Bovans-non-cage and LSL-non-cage. These results indicate that using information from related hybrids can slightly increase the GEBV accuracy. Including purebred information increased the accuracy of hybrid GEBV. When data of hybrid-housing combinations and pure lines were analysed simultaneously, there was a gain in the accuracy of hybrid GEBV, 3–6 points above the accuracy from combining only hybrid information, and 3–14 points above the single trait accuracy. These results indicate the purebred data could be relevant to the predictions of hybrids. Including the hybrid data makes the GEBV accuracy of pure lines much worse compared to using only purebred data. Combining data of pure lines WL and RIR resulted in 9–12 units increase in the GEBV accuracy compared to single trait GEBV of pure lines.

The estimated genetic correlation between BW and tibia strength is moderate (Supplementary Table S1). When we used a bivariate GBLUP, treating BW and tibia strength within-line as correlated genetic traits, the GEBV accuracy increased for hybrids and pure lines (Table 4). For hybrids, the increase in GEBV accuracy was 1 to 40 units above the accuracy from combining hybrids and pure lines data. For pure lines, the increase in GEBV accuracy was 4 units above the single trait accuracy. The raw values of GEBV accuracy without dividing by the square root of heritability are shown in Supplementary Table S2. Within each of the hybrids and pure lines, the standard error of GEBV accuracy is low (Table 4), indicating a low variability of accuracies from the five validation runs. There

Table 3
Estimates of heritability (on the diagonal) and genetic correlation (off-diagonal) ± SE for tibia strength in laying hens under each scenario.

Scenario	Tibia strength traits						
Single trait	Bovans-cage	LSL-cage	Bovans-non-cage	LSL-non-cage	WL	RIR	
6 models, 1 trait in each							
	0.56 ± 0.24	0.11 ± 0.30	0.47 ± 0.28	0.49 ± 0.26	0.22 ± 0.04	0.49 ± 0.05	
Within hybrids across housings		Bovans-cage	Bovans-non-cage		LSL-cage	LSL-non-cage	
2 models, 2 traits in each	Bovans-cage	0.60 ± 0.23		LSL-cage	0.18 ± 0.29		
	Bovans-non-cage	0.78 ± 1.10	0.42 ± 0.28	LSL-non-cage	0.88 ± 1.58	0.50 ± 0.25	
Across hybrids within housings		Bovans-cage	LSL-cage		Bovans-non-cage	LSL-non-cage	
2 models, 2 traits in each	Bovans-cage	0.58 ± 0.24		Bovans-non-cage	0.45 ± 0.16		
	LSL-cage	−0.015 ± 1.6	0.11 ± 0.31	LSL-non-cage	0.99 ± 0.22	0.54 ± 0.17	
Across hybrids across housings		Bovans-cage	LSL-cage	Bovans-non-cage	LSL-non-cage		
1 model of 4 traits	Bovans-cage	0.59 ± 0.21					
	LSL-cage	−0.02 ± 1.3	0.23 ± 0.18				
	Bovans-non-cage	0.66 ± 0.89	0.57 ± 1.4	0.46 ± 0.15			
	LSL-non-cage	0.32 ± 0.45	0.79 ± 0.92	0.90 ± 0.19	0.59 ± 0.16		
Across hybrids across housings + WL		Bovans-cage	LSL-cage	Bovans-non-cage	LSL-non-cage	WL	
1 model of 5 traits	Bovans-cage	0.64 ± 0.16					
	LSL-cage	0.03 ± 0.60	0.39 ± 0.16				
	Bovans-non-cage	0.61 ± 0.69	0.26 ± 0.48	0.51 ± 0.13			
	LSL-non-cage	0.29 ± 0.52	0.65 ± 0.38	0.84 ± 0.15	0.66 ± 0.16		
	WL	0.04 ± 1.52	0.69 ± 0.38	−0.27 ± 0.29	0.05 ± 0.30	0.18 ± 0.06	
Across hybrids across housings + RIR		Bovans-cage	LSL-cage	Bovans-non-cage	LSL-non-cage	RIR	
1 model of 5 traits	Bovans-cage	0.47 ± 0.16					
	LSL-cage	0.09 ± 0.76	0.16 ± 0.13				
	Bovans-non-cage	0.58 ± 0.23	0.68 ± 0.60	0.37 ± 0.11			
	LSL-non-cage	0.28 ± 0.31	0.83 ± 0.45	0.93 ± 0.05	0.50 ± 0.08		
	RIR	−0.07 ± 0.33	0.80 ± 0.44	0.75 ± 0.12	0.93 ± 0.05	0.37 ± 0.05	
Across hybrids across housings + WL + RIR		Bovans-cage	LSL-cage	Bovans-non-cage	LSL-non-cage	WL	RIR
1 model of 6 traits	Bovans-cage	0.50 ± 0.13					
	LSL-cage	0.15 ± 0.35	0.28 ± 0.12				
	Bovans-non-cage	0.57 ± 0.19	0.27 ± 0.28	0.40 ± 0.10			
	LSL-non-cage	0.31 ± 0.25	0.59 ± 0.21	0.86 ± 0.06	0.54 ± 0.08		
	WL	0.02 ± 0.29	0.64 ± 0.21	−0.23 ± 0.19	0.11 ± 0.19	0.16 ± 0.06	
	RIR	0.10 ± 0.29	0.65 ± 0.23	0.57 ± 0.15	0.85 ± 0.07	0.49 ± 0.17	0.39 ± 0.04
Across WL and RIR		WL	RIR				
1 model of 2 traits	WL	0.16 ± 0.03					
	RIR	0.89 ± 0.02	0.39 ± 0.03				

Abbreviations: Bovans = White Bovans hybrid; LSL = Lohmann Selected Leghorn Classic hybrid; WL = White Leghorn; RIR = Rhode Island Red.

Table 4
Accuracy of GEBVs (from cross-validation) ± SE for tibia strength in pure lines and hybrids of laying hens, analysed under single- and multitrait scenarios, including a scenario with BW and within-line tibia strength as correlated genetic traits.

	Scenarios									n
	Single-trait	Multitrait								
Tibia Strength Classes		Within hybrid across housing	Across hybrid within housing	Across hybrid across housing	Across hybrid across housing + WL	Across hybrid across housing + RIR	Across hybrid across housing + WL + RIR	Across WL and RIR	Bivariate of tibia strength + BW	
Bovans-cage	0.29 ± 0.06	0.27 ± 0.07	0.29 ± 0.06	0.29 ± 0.06	0.26 ± 0.07	0.32 ± 0.07	0.31 ± 0.07		0.42 ± 0.08	218
LSL-cage	0.18 ± 0.08	0.19 ± 0.05	0.18 ± 0.08	0.19 ± 0.05	0.22 ± 0.06	0.23 ± 0.04	0.25 ± 0.05		0.65 ± 0.04	213
Bovans-non-cage	0.31 ± 0.03	0.29 ± 0.05	0.37 ± 0.05	0.35 ± 0.04	0.35 ± 0.03	0.41 ± 0.04	0.40 ± 0.03		0.43 ± 0.07	197
LSL-non-cage	0.23 ± 0.1	0.25 ± 0.13	0.29 ± 0.09	0.31 ± 0.12	0.30 ± 0.13	0.37 ± 0.12	0.34 ± 0.13		0.56 ± 0.06	214
WL	0.51 ± 0.02				0.07 ± 0.03		0.05 ± 0.03	0.63 ± 0.02	0.55 ± 0.02	947
RIR	0.69 ± 0.03					−0.46 ± 0.04	0.42 ± 0.03	0.78 ± 0.03	0.73 ± 0.02	924

Abbreviations: GEBVs = Genomic estimated breeding values; Bovans = White Bovans hybrid; LSL = Lohmann Selected Leghorn Classic hybrid; WL = White Leghorn; RIR = Rhode Island Red.

Table 5
Dispersion bias (b1) of GEBVs (from cross-validation) ± SE for tibia strength in pure lines and hybrids of laying hens, evaluated under single- and multitrait scenarios, including a scenario with BW and within-line tibia strength as correlated genetic traits.

	Scenarios									n
	Single-trait	Multitrait								
Tibia Strength Classes		Within hybrid across housing	Across hybrid within housing	Across hybrid across housing	Across hybrid across housing + WL	Across hybrid across housing + RIR	Across hybrid across housing + WL + RIR	Across WL and RIR	Bivariate of tibia strength + BW	
Bovans-cage	1.12 ± 0.29	0.86 ± 0.28	1.12 ± 0.26	0.94 ± 0.26	0.96 ± 0.31	0.97 ± 0.29	0.97 ± 0.28		0.97 ± 0.22	218
LSL-cage	1.21 ± 1.61	0.64 ± 0.47	1.26 ± 1.59	0.67 ± 0.39	0.78 ± 0.33	0.68 ± 0.32	0.77 ± 0.31		1.02 ± 0.07	213
Bovans-non-cage	1.35 ± 0.18	0.95 ± 0.26	1.32 ± 0.31	1.06 ± 0.24	1.09 ± 0.17	1.09 ± 0.19	1.09 ± 0.16		1.07 ± 0.25	197
LSL-non-cage	1.08 ± 0.67	1.13 ± 0.74	1.15 ± 0.61	1.18 ± 0.65	1.19 ± 0.67	1.09 ± 0.57	1.06 ± 0.57		1.06 ± 0.19	214
WL	1.01 ± 0.1				0.21 ± 0.19		0.14 ± 0.2	1.02 ± 0.09	1.01 ± 0.08	947
RIR	1.05 ± 0.07					−3.59 ± 0.53	3.84 ± 0.46	1.27 ± 0.09	1.06 ± 0.06	924

Abbreviations: GEBVs = Genomic estimated breeding values; Bovans = White Bovans hybrid; LSL = Lohmann Selected Leghorn Classic hybrid; WL = White Leghorn; RIR = Rhode Island Red.

Table 6
Mean bias (b_0) of GEBVs (from cross-validation) ± SE for tibia strength in pure lines and hybrids of laying hens, evaluated under single- and multitrait scenarios, including a scenario with BW and within-line tibia strength as correlated genetic traits.

Tibia Strength classes	Scenarios								n
	Single-trait	Multitrait							
		Within hybrid across housing	Across hybrid within housing	Across hybrid across housing	Across hybrid across housing + WL	Across hybrid across housing + RIR	Across hybrid across housing + WL + RIR	Across WL and RIR	Bivariate of tibia strength + BW	
Bovans-cage	−0.01 ± 0.01	−0.01 ± 0.01	−0.01 ± 0.02	−0.02 ± 0.01	0.06 ± 0.03	0.06 ± 0.02	0.01 ± 0.01		0.01 ± 0.06	218
LSL-cage	0.01 ± 0.01	0 ± 0.01	0.01 ± 0.01	0 ± 0.01	0.06 ± 0.04	−0.24 ± 0.11	−0.13 ± 0.05		0.01 ± 0.04	213
Bovans-non-cage	−0.01 ± 0.01	0 ± 0.01	−0.06 ± 0.03	−0.03 ± 0.02	−0.01 ± 0.04	−0.6 ± 0.12	−0.22 ± 0.06		−0.01 ± 0.05	197
LSL-non-cage	−0.02 ± 0.02	0 ± 0.04	0.03 ± 0.04	0.04 ± 0.06	0.1 ± 0.06	−0.92 ± 0.48	−0.35 ± 0.2		0 ± 0.08	214
WL	0 ± 0.01				−0.02 ± 0.02		−0.02 ± 0.03	−0.41 ± 0.03	−0.01 ± 0.03	947
RIR	0 ± 0.01					0.3 ± 0.05	0.41 ± 0.07	0.48 ± 0.07	0 ± 0.02	924

Abbreviations: GEBVs = Genomic estimated breeding values; Bovans = White Bovans hybrid; LSL = Lohmann Selected Leghorn Classic hybrid; WL = White Leghorn; RIR = Rhode Island Red.

was no evidence of upward bias of the GEBV, but a few scenarios showed a downward bias (Tables 5–6). All b_0 (mean bias) tend to be zero or even negative, and b_1 (dispersion bias) are close to one.

Discussion

In the current study, we aimed to obtain GEBV for tibia strength based on different sources of information, using data from two types of commercial layers housed in two housing systems and two pure lines. Hybrid data slightly improved the GEBV accuracy of other hybrids, but not that of pure lines. Pure line data also improved the GEBV accuracy of hybrids above that of combining hybrid information. Combining data from two pure lines improved the GEBV accuracy of both. Combining data across hybrids from different breeding organisations may be unlikely to happen in practice, but it resembles the situation of combining data of different hybrids within the same breeding organisation. In this section, we will discuss: 1) Similarity in the genetic architecture of bone strength and linkage disequilibrium between populations, 2) implications for GEBV of bone quality, 3) the modelling of BW 4) limitations of the current study.

Patterns of linkage disequilibrium across populations

The principal component and linkage disequilibrium analyses are consistent with what is known about layer breeds. The breeding lines that give rise to the white hybrids share common ancestors going back to poultry breed formation, whereas brown layers like the Rhode Island Red are further separated. The similar patterns of LD across the white hybrid populations indicate similar genetic make-up, suggesting the potential success of genomic prediction across these populations. Surprisingly, the two white hybrids from different breeding companies had LD phase persistency around ~0.55–0.60 correlation, higher than with the pure white line. These can be compared to correlations around 0.9 (Fu et al., 2015) and 0.95 between related lines of broilers [41] and 0.8 for the same dairy cattle breed in different countries (de Roos et al., 2008). As bone phenotypes involve invasive or imaging techniques and the extensive genotyping of hybrids is unlikely, the reference population for hybrid bone traits is expected to be small to medium sized. However, as long as they are related, i.e. small but related reference populations, then combining them into a joint reference population could improve the genomic GEBV accuracy (Marjanovic et al., 2021; Wientjes, 2016; Zhou et al., 2014).

Implications for genomic prediction of bone quality

The goal of genomic prediction is to get accurate GEBV of pure lines for hybrid traits, and doing so may require data from hybrid birds. Several strategies have been suggested (reviewed by Duenk et al. (2021)), using a reference population consisting of pure breeding lines, commercial hybrids, or both. In the absence of strong heterosis and genotype-by-environment or genotype-by-genotype interaction for bone quality, selection within pure line is a sensible baseline strategy. In that case, the genetic correlation between breeding lines and hybrids approaches one, and there is no need to collect information about hybrids. Otherwise, there may be a need to collect phenotypes from hybrids, as shown from previous simulations (González-Diéguez et al., 2020; See et al., 2020).

It is an open question that cannot be answered with the present data on how accurate the marker effects estimated from hybrid GEBVs would be for predicting pure line birds for hybrid performance. However, we hypothesise that different hybrids and pure lines can be pooled in a combined reference population with the

help of multitrait analysis. The gains in this study were limited, but so were the population sizes. Perhaps the hybrids are not genetically close enough to the pure lines in the current study, to give a notable improvement when combining data. A previous study indicated that combining large datasets of related lines may result in only slight or no improvement in GEBV accuracy (Calus et al., 2014; Simeone et al., 2012). The sample size is also limited. For these reasons, it is a challenge to estimate genetic correlations between the pure lines and the hybrids, consequently, pure line data may introduce noise to the predictions.

Modelling of BW

There are two conceptually different approaches to handle the known relationship between bone–breaking strength and BW in genomic prediction. BW has a weak to moderate genetic correlation with bone traits (Dunn et al., 2021; Bishop et al., 2000), consistent with the current findings. In genetic mapping studies, modelling BW as a fixed covariate may be useful to identify genes associated with bone mineralisation separate from genes of BW (Sallam et al., 2023; Johnsson et al., 2022; Raymond et al., 2018; Schreiweis et al., 2005). In the context of poultry breeding, it perhaps makes more sense to model tibia strength unadjusted, while also estimating breeding values for BW in a multitrait model. Genomic breeding values for BW and tibia strength can be then included in a selection index with appropriate weights. The GEBV accuracy for unadjusted tibia strength and BW within lines in multitrait models had higher accuracy than for adjusted BW. Genome-wide association studies in these hybrids have (Johnsson et al., 2022) identified major loci for BW, and these large segregating effects may make accounting for BW more important in the hybrids than within pure lines.

Limitation of the current study

Hybrids have small data size in the current study and are not the direct grand-offspring of the current pure lines. Therefore, the reported estimates of genetic correlations between hybrids and pure lines are noisy and may deviate from those within breeding companies with direct pedigree links. The current estimates of genetic correlations between pure lines and hybrids are not strong, in agreement with the literature reviewed by Calus et al. (Calus et al., 2023). The current estimates of genetic correlation also suggest that genes of tibia strength differ between either cage and non-cage housings, or hybrids and pure lines. However, the uncertainty is too great to draw any definite conclusions. Finally, this study is based on post-mortem phenotyping. Measuring tibia on live birds (Sallam et al., 2024; Wilson et al., 2022) instead may help to obtain easier phenotyping of the selection candidates.

Conclusions

The results suggest that genomic prediction of bone quality in laying hens is possible, and that there is some potential for sharing of information between closely related pure lines and hybrids. The maximum GEBV accuracy obtained for tibia strength ranged from 0.42 to 0.65 for hybrids and from 0.63 to 0.78 for pure lines.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101452) can be found at the foot of the online page, in the Appendix section.

Ethics approval

Not applicable, since no new data on animals were collected in this study.

Data and model availability statement

For the breeding line (RIR and WL), the datasets analysed during the current study are not publicly available. For hybrids (Bovans White and Lohmann Selected Leghorn Classic), the underlying data have been previously deposited on Figshare (https://doi.org/10.6084/m9.figshare.14405894). Information can be made available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare that they have no competing interests with the exception of BA and MS who are employees of Lohmann Breeders.

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