Transmission of an environmental modification in quails across three generations: changes in phenotypic variance components

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ABSTRACT

While epigenetic variations can contribute to shaping phenotypic diversity, it can be challenging to isolate and quantify the portion of trait variability under epigenetic influence. In this study we compared the phenotypic responses for different traits of two epilines of Japanese quails (*Coturnix japonica*) across three generations, using a large sample size. These epilines were built in parallel following (epi +), or not (epi -), an initial genistein ingestion in the ancestors' diet and were maintained to harbour a similar genetic structure. Linear models were fitted to extract the fraction of variance allocated to multiple factors such as family, sex and epiline. The latter was found to be significantly associated with body weight. The proportion of phenotypic variance explained by the epiline progressively increased from the first generation (G0) to the last (G2), leading - for example in body weight at slaughter - to an average difference for adult males and females in G2 of 9 grams and 14 grams respectively, between epi + offspring and controls (epi-). Although these findings suggest genetic drift, they could also reveal a possible transgenerational effect of the initial diet disruption. The analysis of variance (ANOVA) of other phenotypes did not display a significant effect of the epiline. This innovative experimental design offered a unique opportunity to better understand the evolution of phenotypic variance and the parameters constituting it across three generations following or not an environmental change.

Key words : Epigenetic, Phenotype, Environmental modification, Transgenerational, Multigenerational, *Coturnix japonica*

INTRODUCTION

The increased frequency of environmental constraints due to climate change (e.g. heat waves, resource limitation ...) can be a source of stress for farm animals. One of the key drivers of

adaptation is the emergence of new phenotypes, which arise from the combined effects of genetic influence of parental alleles and non-genetic factors. Among those non-genetic factors, epigenetic phenomena, such as DNA methylation or histone modifications, can be involved in the construction of phenotypes (Duncan et al., 2014). Fluctuations in the parental environment can trigger epigenetic modifications, resulting in changes in gene regulatory mechanisms and thus gene expression, even in the progeny (Guerrero-Bosagna et al., 2018). Understanding the impact and transmission of environmental effects to subsequent generations is crucial to comprehend how animals adapt to changing environments to maintain animal production needs while ensuring animal welfare.

Recently, the impact of the parental epigenome on offspring's developmental phenotypes has been a research topic of growing interest in different livestock species like cattle (Kiefer et al., 2021), pig (Wu et al., 2024), sheep (Fonseca et al., 2023) and poultry (Hu et al., 2020). Even if most studies tackle the direct impact of the environment on the traits of exposed animals and on the performances of the subsequent generation, a handful have managed to evaluate the possible transgenerational inheritance of epigenetic phenotypes: as defined by Skinner 2008, we consider an environmental effect to be transgenerationally transmitted when the individuals exhibiting trait variations were not exposed to the environmental stressor, even as germ cells. On the contrary, an effect is considered as intergenerationally transmitted when trait variations are observed in the offspring of the individuals exposed to an environmental modification, therefore in an individual exposed as embryo or germ cells status. Multigenerational, including inter and transgenerational, studies in farm animal are challenging due to the need to raise multiple generations. To this end, quail is an ideal avian model for multigenerational analysis, thanks to its short generation interval, the wide range of measurable phenotypes, and the controlled breeding practices and limited space in which it can be reared. Previous studies on a variety of traits such as body weight, egg production and

behavioural characteristics using Japanese quails as model organisms have reported multi and transgenerational phenotypic responses of the progeny after a thermal increase in the parental embryonic environment (Vitorino Carvalho et al., 2023), after in-ovo injection of genistein, an endocrine disruptor (Leroux et al., 2017), and after exposure to maternal stress (Charrier et al., 2024). These studies suggest a possible transmission of environmental effects through changes occurring in the prenatal embryonic environment. In addition, some evidence of the importance of grandmother's diet was documented in mule and Muscovy ducks, with significant variations in ducklings and adult weights after methionine deprivation in the diets of the grandmothers (Brun et al., 2015). This suggests that diet disruptions can indeed be a cause of phenotypic response to an unknown yet memorised environment, through a specific inheritance mechanism.

In the present study we used genistein as the environmental stressor in the ancestor diets of Japanese quails. Genistein is a phytoestrogen compound naturally present in soy, and was notably associated with significant shifts in methylation profiles in the promoter region of the Agouti gene in mice (Dolinoy et al., 2006). Although this molecule offers promising results in cancer treatment (Rasheed et al., 2022; Casari et al., 2025), soy overconsumption has demonstrated harmful effects on reproductive (Liu et al., 2020; Lozi et al., 2021; Rashid et al., 2022; Toktay et al., 2020) or cognitive (Svensson et al., 2021) functions. These effects of endocrine disruptors on health seem to be persistent over several generations, although this remains relatively unexplored, despite a few specific studies (Brulport et al., 2021). In addition, the use of genistein as supplementation within the feed of Japanese quails has been shown to lead to better adaptation of birds to heat stress by stimulating feed efficiency and growth rate (Onderci et al., 2011).

While epigenetic variations can contribute to shaping phenotypic diversity in offspring, isolating and quantifying the portion of trait variation influenced by epigenetics remains

challenging. To date, although genetic contribution has largely been documented for a wide range of traits in a large number of poultry species, no direct quantification of the epigenetic influence on these traits has been reported. The environment usually accounts for a large part of the observed variability depending on the phenotypes measured, which translates to low heritabilities even under selection (Gaya et al., 2006, Prince et al., 2020; Chomchuen et al., 2022; van Oers et al., 2003; Wright and Henriksen, 2020; Lormant et al., 2020) providing a compelling reason to quantify the variance portion explained by non-genetic factors, including epigenetics. In this study, a case - control design allowed to build two distinct epilines. The epiline information provides an indirect way to estimate the epigenetic contribution to the trait. By investigating the multigenerational transmission of the effects of an initial diet disruption across three generations of quail, we aimed to capture the inter- and transgenerational impacts of environmental influences, likely mediated by epigenetic mechanisms. The portion of phenotypic variability linked to an ancestor's environmental change and its evolution across generations was quantified in body weights.

MATERIALS AND METHODS

Quail mating design

All animals in the experiment come from a quail experimental line (HSR, for High Social Reinstatement) arising from a divergent selection on social motivation (Mills and Faure, 1991). From this common genetic background, twenty initial founding families were crossed, producing the generation G-1. In each founding family, two full sib females were mated at maturity with the same male, originating from another family. One of the females was administered a genistein capsule (100 mg, Molekula group) for thirty days after mating while the other one was only provided with empty capsules, leading to the development of G0 individuals in two different embryonic environments. The females that received the genistein

are the dams of the treated epiline (thereafter called epi+), in opposition to the control epiline (epi-). After this initial diet disruption, the quails were mated following a mirror design, with males and females from the same founding families mated in each line, to maximize the genetic balance between the two epilines. Groups were then maintained under standard rearing conditions, at the breeding facility, for the three subsequent generations (G0, G1 and G2), with water and feed *ad libitum* (Fig.1). From hatching to 49 days-of-age, individuals were floor-raised on litter altogether, in climate-controlled housing. Animals were then placed within cages of two individuals of same sex, until the age of twenty weeks, when they were coupled for mating. All diet, temperature and light conditions were standard and the same for all animals, all reared in the same barn.

Phenotypic traits

Phenotypic records were collected over four generations for body weights, tissue weights, egg production and behavioural response to isolation.

Body weights (BW). Body weights were measured at different developmental stages: one week, four weeks, seven weeks and slaughter (thirty weeks of age on average) for all quails. A unique scale was used for all measurements and weights were collected at the same age when possible to ensure consistency between generations. Slaughter was done through electronarcosis immediately following the recording of the final body weight measure.

Tissue weights (TW). Several tissues were weighed: abdominal fat (for all generations), heart, liver (for G0 onwards), and testicle (for G1 onwards). All weights were adjusted by correcting for the weight of the quail at slaughter.

Egg production (PROD). At twenty weeks of age, quails were placed in cages for reproduction. Egg production data, total egg number during the laying period and precocity,

were recorded from the first day in the cage for 49 days. The laying rate was estimated as the number of eggs laid over the laying period. The mean egg weight per quail was calculated on a basis of four eggs collected and weighed per quail.

Behaviour at isolation (BHV). Adult quails underwent an open field behavioural test in which their reactivity to a new environment was assessed. Each quail was placed in a cubic arena (80 cm) of white wood with a floor made of beige linoleum, in an unknown experimental room surrounded by an unknown white curtain. Each quail was individually placed at the centre of the arena and allowed to freely explore it for 300 seconds. For each trial, the time spent in the inner area (a square of 20 cm × 20 cm at the centre of the arena), the frequency of time spent at the periphery (outside the inner area) as well as the total distance covered by the quail was recorded by a camera placed above the arena. The open field test was performed for all generations.

Pairwise differences between epilines

One-way ANOVA was applied to compare the effect of epilines on the raw phenotypes. Given the strong sexual dimorphism in quails, the test was applied to all raw phenotypes in each sex and generation separately. The null hypothesis of H0 “No significant effect of the epiline on the phenotype” was rejected at the 5% level of significance using a F-test. A Tukey HSD (Honestly Significant Difference) test was then conducted to estimate pairwise differences between epilines for each phenotype with a statistical significant epiline effect using the R ‘rstatix’ package (version 0.7.2).

Variance components estimation for body weight traits

Following the results of pairwise ANOVA we decided to focus or analyses on body weight traits. To estimate the percentage of variance explained by influential factors such as the family, sex and epiline, linear models were fitted to normalized body weights.

Data normalization. To minimize skewness and meet the assumptions of linear modeling, phenotypes were normalized to minimize skewness using the R 'moments' package (version 0.14.1). Skewness of Log_{10}, square root and inverse transformed data were compared to that of the raw data and the transformation associated with the lowest skewness value was subsequently retained for each trait.

Linear models (LM). For each body weight at each generation, the optimal set of parameters was first determined with a stepwise model selection using the stepAIC function of the R 'MASS' package (version 7.3-65). A linear model was fitted for each trait as follows:

$$Y = \mu + \boldsymbol{X}\beta + \varepsilon$$

where Y is the phenotype, μ is the overall mean, $\boldsymbol{X}$ is the design matrix for the fixed effects, β is the coefficient vector of fixed effects and ε is the residual. Fixed effects included in the model were sex (male, female), epiline (epi-, epi+) and family (origin from founders) and their pairwise interactions (sex:epiline, sex:family and epiline:family). Effects were iteratively added or deleted until the model with the lowest Akaike information criterion (AIC) was identified for each trait.

Cross Validations. After selecting the optimal model, we evaluated the fit of the model using 100 random fold cross validations (80% of data in the Train set, 20% in the Test set). For each fold, the Train set was used to fit the model using the optimal equation determined with StepAIC, and it was then used to predict the phenotypic response of the Test set. Correlations between observed and predicted Test phenotypes were then assessed. The

coefficient of determination R^2, which represents the overall variance of the trait captured by the model, was computed for each fold using the R squared of the linear model.

Variance components. The proportion of variance explained by each factor for a given trait was estimated using an analysis of variance (ANOVA – type III). The total sum of squares SS_{Total} is decomposed into the sum of squares for each factor j SS_{Factor_j} and residual variance SS_{res}. The proportion of variance explained (PVE) of factor j may be calculated as:

$$PVE_j = \frac{SS_{Factor_j}}{SS_{Total}}$$

Estimations were performed using the R package 'FactoMineR' (version 2.12) with Type III sum of squares. We ensured success in variance components estimations by retaining only families with sufficient number of quails per epiline.

All statistical analyses were performed using R 4.5.0 (R core team).

RESULTS

The summary statistics of traits collected from G0 onwards are available in Table 1. The summary statistics of traits collected in G-1 are included in Supplementary Table S1.

Impact of the epiline on phenotypes and evolution across generations

Body weights (BW). Significant differences between epilines were observed across several body weights. Females showed significant differences starting from generation G1 at all ages with epi+ quails systematically weighing more on average than controls epi-. The same pattern was found in males where the groups began to consistently diverge in G2 at all ages; however, no significant difference was observed in earlier generations, with the exception of G0 at four weeks of age (Fig. 2).

Differences in average BW at all ages became increasingly more pronounced over the generations. In G0, the difference between epi+ and epi- at slaughter was on average 4 grams for both sexes, which was not found to be significant. In G1, the difference between epilines for females was on average 7 grams and reached 14 grams in G2. In males, a similar pattern was observed in G2, with epi+ quails weighing 9 grams more on average at slaughter whereas a maximum difference of 2 grams was observed in earlier generations. The growth curve to highlight this longitudinal evolution is added in Supplementary Figure S1.

Other phenotypes. For other phenotypes, such as tissue weights (TW) , egg production (PROD) or behaviour at isolation (BHV), significant differences between epilines were sporadic and sometimes linked to individual singularities. These results are displayed in Supplementary Figure S2-S4.

Body weight variance explained by parameters: evolution across generations

Effect of the epiline. Model fitting revealed that the epiline explained a different portion of the variability observed in BW according to the generation and age of the quails. The epiline effect was found significant from four-weeks old quails onwards, in all generations. First, at one week of age, results showed significance in G2 only, with the epiline explaining 7.6% of the variability. Then, for four- and seven-weeks old animals, the epiline explained a negligible amount of weight variability in G0 (under 1%), but it was found to increase in later generations, accounting for around 2% in G1, to finally reach a more substantial proportion in G2 (over 15%) at those ages. Finally, the portion of variance linked to the epiline at adult age (BW at slaughter) was much lower than at previous ages (less than 1% in G0 and G1 and 4.5% in G2) (Table 2, Fig.3).

Effect of other factors. Contrary to the increase of the epiline effect across generations, the portion of phenotypic variance explained by the family declined, starting, at one week of age, from 46% in G0 to 23% in G1 and decreasing again to 14% in G2 (Table 3). The family effect was also stronger in younger animals. It ranged from 46% to 9%, from 23% to 3.5%, and from 14.4% to 5% between one-week of age and slaughter for G0, G1 and G2 respectively. On the other hand the proportion of variability explained by the sex always increased with animal age. It ranged from 0.5% to 67%, from 1.4% to 70.5% and from 1.4% to 63.4% between one-week of age and slaughter for G0, G1 and G2 respectively. The interaction parameter, when conserved, always involved the epiline and the family, except in two traits: in body weight at one week in G1 where it concerned epiline and sex, and in body weight at slaughter in G0 where it concerned epiline and sex as well as epiline and family. Depending on the generation and the trait, this parameter showed little to moderate effect in explaining the phenotypic variance, ranging from 0 to 12%. Overall, the residual remained high in all traits but BW at slaughter, regardless of the generation.

Cross validations. The predictive performance of the models, evaluated using cross validations, varied considerably across BW traits. Body weight at slaughter consistently exhibited the highest prediction accuracy across all generations ($R^2 \geq 0.8$, Corr ≥ 0.8), highlighting good model performance for adult individuals. In contrast, for younger animals, the coefficients of determination were significantly lower ($R^2 \leq 0.5$ in G0 and G1 and $R^2 \leq 0.6$ in G2). The correlations between Train and Test sets were also lower, ranging between 0.4 and 0.6, suggesting limited predictive power of our models for these traits; these results are in line with the large residual variance components estimated (Table 4).

DISCUSSION

The aim of this study was to analyze phenotypic variations across three generations of quails following supplementation with genistein, an endocrine disruptor. For this purpose we built one of the largest available design in livestock containing groups impacted (treatment) or not (control) by environmental stressors and multiple generations of mirror breeding to limit the impact of genetic variation in the determination of trait changes through time.

Genistein ingestion is associated with different phenotypic responses across generations

In both sexes, body weight (BW) tended to increase in the treated epiline (epi+) compared to the control epiline (epi-), with the difference between epilines becoming progressively more pronounced from generation G0 to G2. In a pilot study (Leroux et al 2017), a similar pattern was observed in G2 females: genistein injection into the egg was associated with increased BW at three weeks of age. However, in that study, the effect was reversed in males (genistein injection associated with decreased BW). In the present study, we did not observe such sex-specific interactions regardless of the generation or age considered. On the other hand, the results obtained here concur with other studies regarding the significant impact of genistein on BW. The scientific community remains divided on the impact of the phytoestrogen on the organism (Xiang et al., 2023; Rockwood et al., 2019), with most studies tackling the direct effect of ingestion on the concerned individuals.

Production (PROD) traits did not exhibit the strong epiline-related effects seen previously in the pilot study, where epi+ females began laying eggs more than eight days later than control females on average. Likewise, abdominal fat weight and behaviour (BHV) traits did not demonstrate the previously reported significant differences between epilines. The discrepancies between the two studies, despite using the same quail genetic line and the same endocrine disruptor, are likely attributable to differences in the method of genistein administration: in the pilot study, 500 µg of genistein were directly injected into the egg

before incubation, whereas in the present study genistein was administered orally to the G-1 mother to mimic more realistic breeding conditions, resulting in substantially lower concentrations of the molecule in the egg (Lin et al., 2004).

Overall, results reveal different sensitivities of the phenotypes towards genistein ingestion. Although this molecule is already used as a supplement to increase BW in broilers (Lv et al., 2018), it has been observed that it affects other phenotypes as well, but not as strongly (Navarro-Guillén et al., 2025).

Missing variability highlights the complexity of phenotypes

The proportion of phenotypic variance explained by the family displayed a clear trend with age, decreasing over the lifetime (with the exception of week 7 in G1). In our model, the family factor was accounted for as a way to include the ancestral genetic effect. This suggests that the influence of the inherited genetic background becomes progressively less determinant as individuals age. Conversely, the contribution of the sex factor increased with age, consistent with the emergence of pronounced sexual dimorphism in quail. These patterns illustrate that different factors influence animal phenotypes in distinct ways at different stages of life, and that the proportion of phenotypic variance explained by a given factor is therefore strongly dependent on the individual's age at the time of measurement.

Nevertheless, residual variance was substantial across all body weights. This is highlighted by the generally weak values of coefficient of determination (R^2), suggesting the difficulty of the models to accurately reflect the overall variability of BW. A potential explanation relies on the complex determinism of phenotypic traits collected, influenced by various environmental factors, some of which the design may have failed to eliminate despite the controlled management. Multiple studies also reported high residual variances remaining, for example on a New Zealand bird species, a study found that home-range sharing and genetic relatedness

among individuals contributed to phenotypic similarities for several morphological traits, yet residual variance remained high (Rutschmann et al., 2020). Similarly, Beziers et al 2019 reported high residual variance for stress-induced corticosterone levels in a barn owl population, hypothesizing an additional factor beyond genetics or nest environment: the experienced environment. Since all individuals from the same generation in our study were reared under identical conditions, a plausible explanation for this residual variance would be the transmission of the experienced environment from the first generation, possibly involving slight variations in genistein dosage among eggs. The embryonic environment is known to influence the individual phenotype later in life, especially in birds (Frésard et al., 2013).

Possible inter and transgenerational transmission of genistein effect

The availability of phenotypic records for all individuals of the design highlighted an overall progressive increase of the epiline effect from G0 to G2 on BW. This phenomenon is most likely due to genetic drift (Falconer and Mackay., 1996, Hospital and Chevalet, 1996), even though, the "mirror" mating design was implemented to minimize genetic effects, ensuring a balanced origin of founders between epilines in each generation.

Alternatively, one could explain such change through an increasing environmental influence across generations. This has been reported in several species, including quail (Vitorino Carvalho et al., 2023), but such effects were generally associated with repeated environmental challenges in each generation. Here, genistein was only administered once and individuals were not selected, suggesting a possible transmission of the environmental effects to the progeny. This hypothesis concurs with results found by Karami et al., 2025 on the same dataset. Indeed, the authors identified a significant difference between expected and estimated sire and dams path coefficients, marking a probable environmental effect on BW traits. Their result supports a potential transmission of environmental effects via non-genetic mechanisms.

While the phenotypic effects of genistein exposure varied in nature and intensity across generations and traits, our findings demonstrate that an environmental exposure during development can induce persistent phenotypic changes, particularly on BW.

Further investigation is needed to understand fully the molecular mechanisms underlying the increased proportion of phenotypic variance explained by the epiline across generations. Although the contribution of genetic drift cannot be fully excluded despite the controlled mating design and the absence of progeny selection, a molecular analysis would help dissect the potential involvement of non-genetic inheritance mechanisms.

To better disentangle these effects, future studies will focus on dissecting genome-wide epigenetic profiling (e.g., DNA methylation) across generations. For the traits showing epiline effect, EWAS (epigenome wide association studies) and metQTL (methylation quantitative trait loci) analyses should be performed to identify genomic regions where epigenetic marks govern phenotypic changes or where genetic variation is driving epigenetic changes. This will help decipher the variance due to genetic and epigenetic mechanisms and molecular pathways involved in its inheritance.

Ethics approval

Animals were bred at the UE1295 PEAT (Nouzilly, France, doi: 10.15454/1.5572326250887292E12) with official authorization for the animals (APAFIS#29977-2021010717073072 v5), and PEAT agreement (D371751).

Data and model availability statement

All data are available on Zenodo, 10.5281/zenodo.15796146 (https://zenodo.org/records/15796146). The code used to run the analysis is available on GitLab: https://forge.inrae.fr/stacy.rousse/transgenerational_pheno_quails

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

The authors did not resort to using AI or AI-assisted technologies for this work.

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Declaration of interest

The authors declare that they do not have financial conflicts of interest in relation to the content of the article.

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Table 1 Summary statistics for all phenotypic traits in different generations from G0 onwards.

Trait		G0						G1						G2					
		Nobs	mean	sd	CV	min	max	Nobs	mean	sd	CV	min	max	Nobs	mean	sd	CV	min	max
BW[1]	1 week	432	28.26	3.06	0.11	17	38	392	25.79	2.87	0.11	16	32	519	25.62	2.86	0.11	17	34
	4 weeks	432	120.94	9.71	0.08	93	152	393	120.93	10.29	0.09	81	162	514	119.63	10.94	0.09	87	153
	7 weeks	431	157.26	10.55	0.07	129	187	392	153.55	12.28	0.08	125	195	508	157.05	12.46	0.08	128	192
	slaughter	398	204.53	28.13	0.14	145	279	373	205.1	32.02	0.16	128	281	414	202.92	31.37	0.15	142	284
TW[2]	heart	135	0.01	0	0.18	0	0.02	370	0.01	0	0.15	0	0.01	408	0.01	0	0.16	0.01	0.02
	liver	161	0.03	0.01	0.23	0.01	0.05	371	0.02	0.01	0.24	0.01	0.04	410	0.02	0.01	0.31	0.01	0.04
	abdo. fat[5]	390	0.01	0	0.75	0	0.02	371	0.01	0.01	0.91	0	0.05	410	0.01	0	0.86	0	0.04
	testicle	-	-	-	-	-	-	173	0.02	0	0.23	0	0.04	207	0.02	0	0.19	0.01	0.03
PROD[3]	precocity	212	74.72	7.26	0.1	65	113	202	81.01	11.15	0.14	69	116	216	76.99	6.65	0.09	67	129
	laying rate	212	0.81	0.19	0.24	0.02	1	202	0.79	0.19	0.24	0.06	1	215	0.82	0.18	0.21	0.04	1
	egg weight	188	0.05	0	0.09	0.03	0.06	189	0.05	0	0.09	0.03	0.07	199	0.05	0	0.09	0.04	0.06
BHV[4]	total distance	404	3362.43	2309.15	0.69	68.59	14968.27	386	2691.85	1769.25	0.66	227.87	8320.07	408	3708.42	2081.7	0.56	334.19	14295.31

duration at centre	404	125.58	83.38	0.66	0.08	300.08	374	113.08	78.67	0.7	0.28	300.04	393	117.13	76.16	0.65	0.04	300.04
freq. at periphery[6]	404	26.23	21.06	0.8	0	159	386	25.76	19.21	0.75	0	99	408	34.76	28.15	0.81	0	242

Nobs = number of phenotypic records; mean; sd = standard deviation; CV = coefficient of variation; min = minimum; max = maximum.

[1] BW = body weights

[2] TW = tissue weights. All tissue weights are adjusted (corrected by the weight at slaughter).

[3] PROD = production traits. Egg weight is adjusted (corrected by the weight at slaughter).

[4] BHV = behavioural traits. Results to an isolation test.

[5] abdominal fat

[6] frequency at periphery

Table 2 Percentage of body weight variance explained by the epiline in each generation.

Trait	G0	G1	G2
1 week	0	0.153	7.641
		[-0.023;0.329]	[6.174;9.108]
4 weeks	0.818	2.984	17.607
	[0.442;1.194]	[2.038;3.930]	[15.684;19.530]
7 weeks	0.264	2.175	16.304
	[0.081;0.446]	[1.550;2.800]	[14.736;17.872]
slaughter	0.262	0.530	4.492
	[0.117;0.407]	[0.321;0.739]	[3.734;5.250]

All figures are percentages and represent the average phenotypic variance explained by the epiline. The confidence interval (brackets) was calculated using the standard deviations across the 100 folds of cross validations.

Table 3 Body weight variability decomposed into the different variance components (in percentage).

Trait	Gen.	Effect	Var	Sd
BW 1 week	G0	epiline	0	0
		family	46,037	1,819
		sex	0,515	0,257
		interaction	0	0
		residual	53,448	1,829
	G1	epiline	0,153	0,176
		family	23,264	1,794
		sex	1,396	0,512
		interaction	0,732	0,441
		residual	74,454	1,777
	G2	epiline	7,641	1,467
		family	14,389	1,449
		sex	1,414	0,552
		interaction	13,17	1,783
		residual	63,386	2,279
BW 4 weeks	G0	epiline	0,818	0,376
		family	31,288	1,912
		sex	4,617	0,881
		interaction	7,119	1,084
		residual	56,158	1,99
	G1	epiline	2,984	0,946
		family	13,513	1,573
		sex	10,322	1,41

		interaction	0	0
		residual	73,181	1,634
	G2	epiline	17,607	1,923
		family	13,761	1,432
		sex	9,146	1,19
		interaction	9,526	1,151
		residual	49,961	1,885
BW 7 weeks	G0	epiline	0,264	0,183
		family	26,363	1,873
		sex	9,315	1,284
		interaction	10,927	1,315
		residual	53,131	2,027
	G1	epiline	2,175	0,625
		family	17,177	1,575
		sex	13,574	1,5
		interaction	12,62	1,291
		residual	54,454	1,837
	G2	epiline	16,304	1,568
		family	10,482	0,92
		sex	14,708	1,621
		interaction	12,691	1,327
		residual	45,815	1,713
BW slaughter	G0	epiline	0,262	0,145
		family	9,026	0,677
		sex	67,148	1,141

	interaction	3,411	0,466
	residual	20,153	1,204
G1	epiline	0,53	0,209
	family	3,479	0,429
	sex	70,513	1,247
	interaction	4,308	0,521
	residual	21,17	1,153
G2	epiline	4,492	0,758
	family	4,917	0,628
	sex	63,378	1,917
	interaction	4,789	0,618
	residual	22,423	2,01

Trait; Gen. = generation; Effect; Var = variance in percentage; Sd = standard deviation in percentage. The estimates were calculated from the extraction of the sum of squares from analyses of variance (ANOVA type III) results. All figures are percentages and represent the average share of phenotypic variability explained by the different factors using the estimates across the 100 folds of cross validations.

Table 4 Model robustness and correlations after cross validations.

Trait	Generation	R^2 av	R^2 sd	Corr av	Corr sd
1 week	G0	0.468	0.0185	0.622	0.0584
	G1	0.292	0.0175	0.434	0.0766
	G2	0.404	0.0212	0.474	0.0708
4 weeks	G0	0.456	0.0203	0.540	0.0768
	G1	0.276	0.0159	0.397	0.0767
	G2	0.545	0.0163	0.646	0.0557
7 weeks	G0	0.485	0.0188	0.603	0.0602
	G1	0.497	0.0185	0.563	0.0718
	G2	0.575	0.0166	0.688	0.0524
slaughter	G0	0.816	0.00997	0.866	0.0276
	G1	0.822	0.00864	0.862	0.0278
	G2	0.806	0.0178	0.860	0.0424

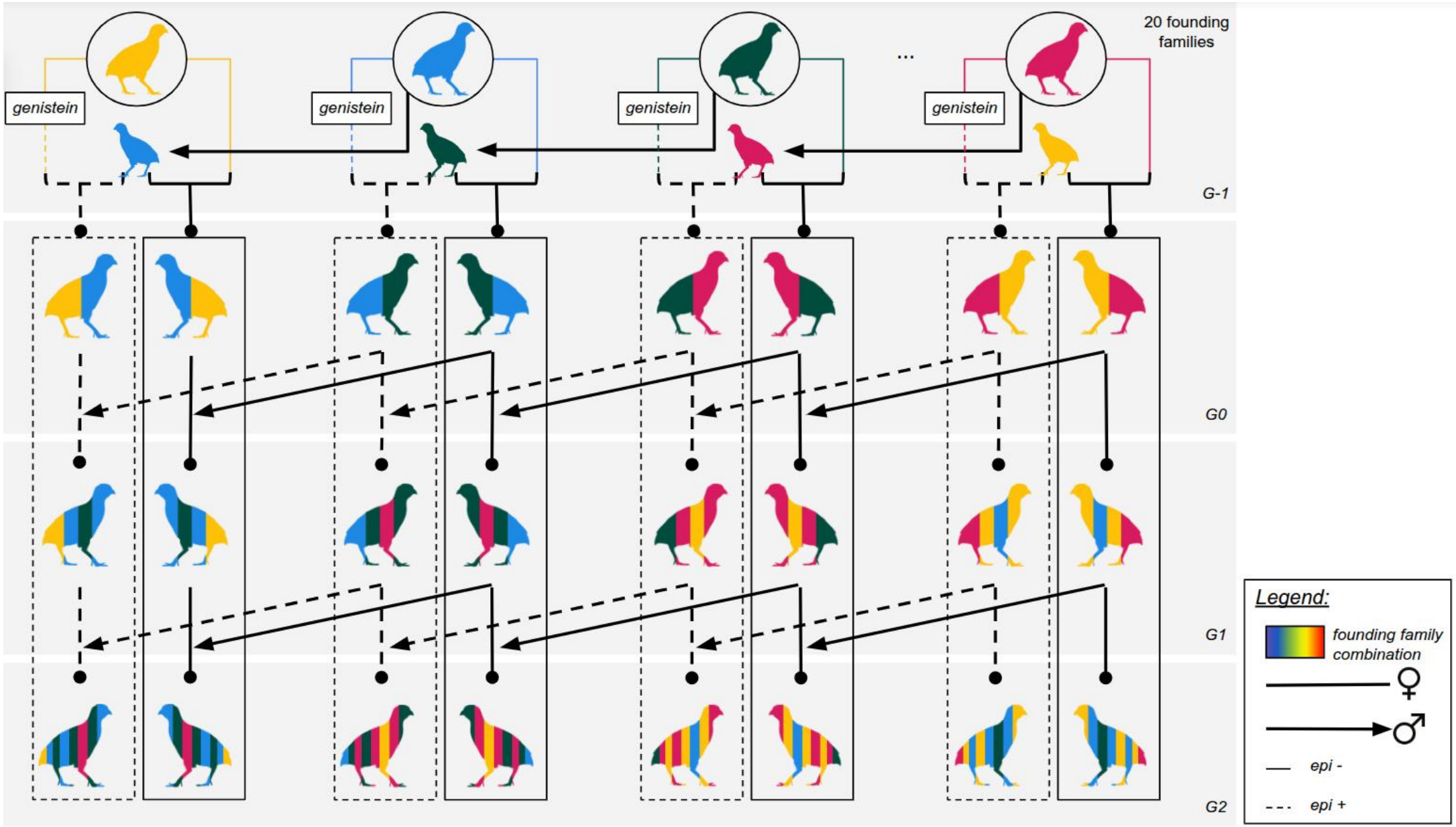
R^2 av: average R^2; R^2 sd: R^2 standard deviation; Corr av: average correlation ; Corr sd: correlation standard deviation. The results were obtained by estimating the model performance across cross validations between observed (real) and predicted data.

Figure captions

Fig. 1. Quail mirror mating design. Genistein capsules were administered to females in G-1 while their full-sib female counterparts were provided empty capsules. Both dams were then mated with the same male coming from another genetic background. The G0 progeny was divided in 2 epilines (epi+ deriving from the dams having ingested the genistein and epi- deriving from the control dams) of similar genetic background. The following matings were controlled to respect the balance of the genetic contribution from founders by ensuring a mirror between epilines (solid lines: epi- and dashed lines: epi+). The genetic contribution of founders is represented by the colors.

Fig. 2. Analysis of variance on body weights at one week, four weeks, seven weeks and at slaughter (thirty weeks) between epilines. The study was conducted for each combination of sex and generation independently. The asterisk indicates the level of significance of the Tukey-HSD test results.

Fig. 3. Phenotypic variance partition (in percentage) between all factors (sex, family, epiline, and their interaction) per generation. The interaction parameter always involved the epiline with either the family, the sex, or both. Body weights are arranged within generations G0, G1 and G2.


20 founding families
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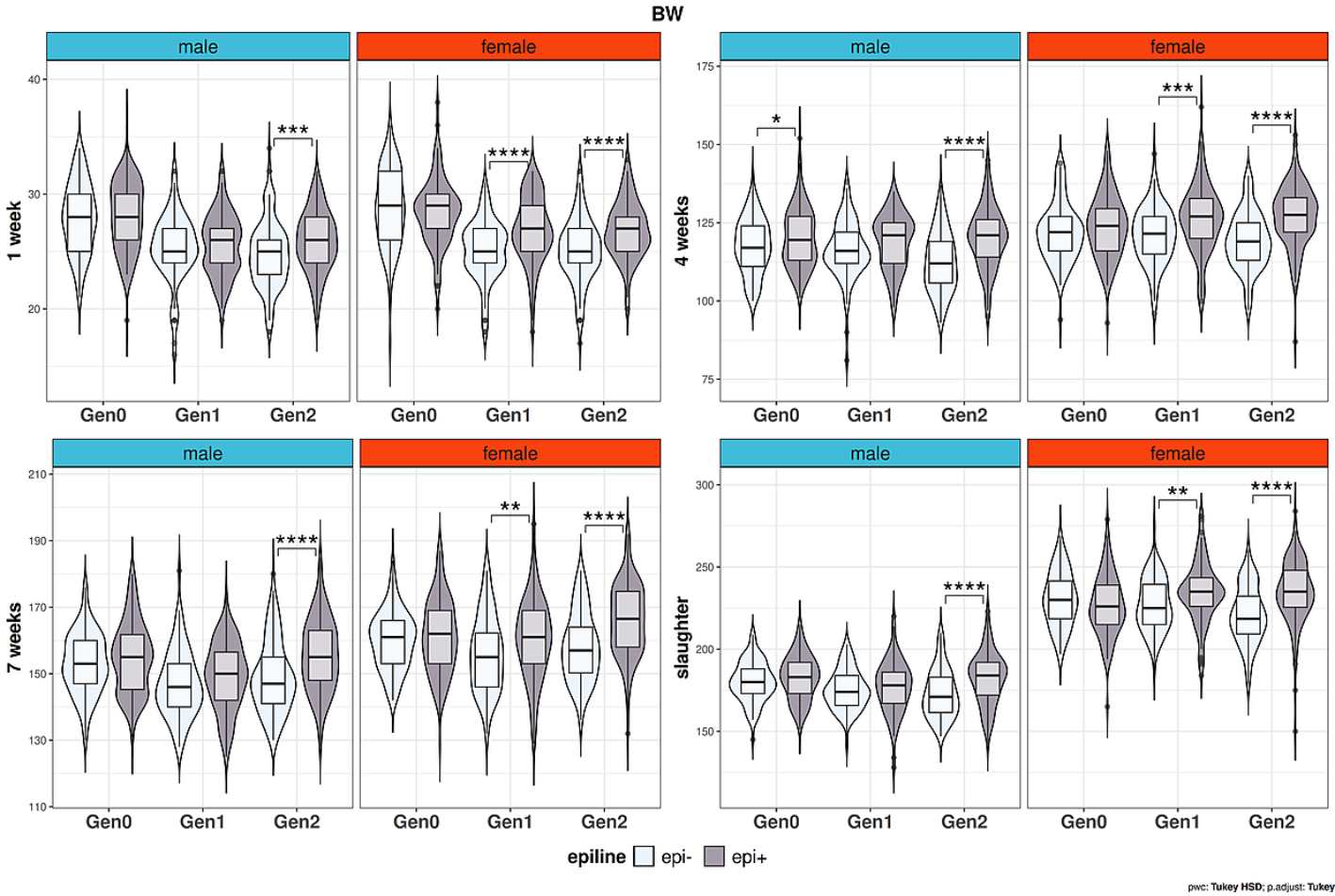
BW
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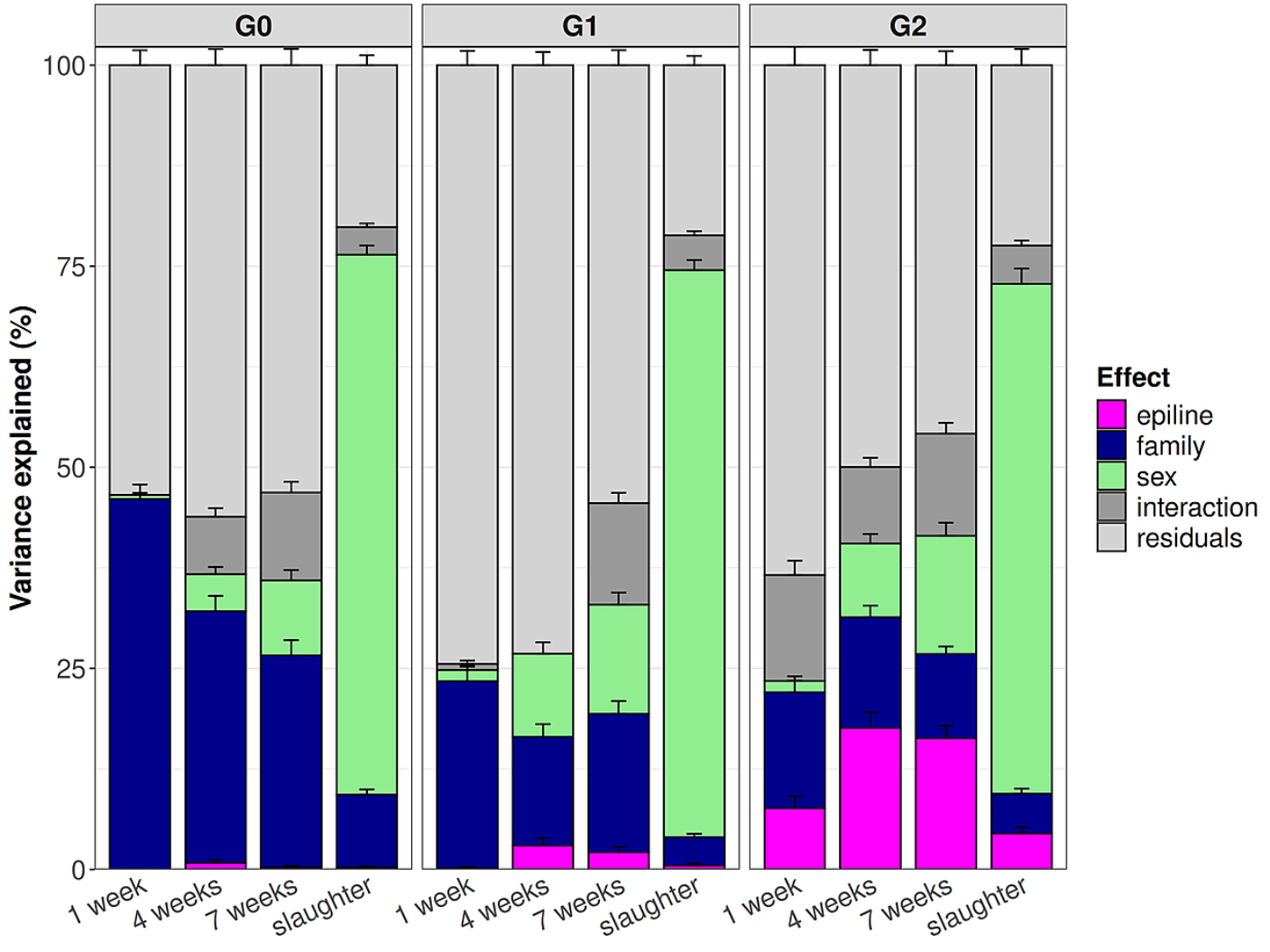
G0
G1
G2
Variance explained (%)
100
75
50
25
0
1 week
4 weeks
7 weeks
slaughter
Effect
epiline
family
sex
interaction
residuals