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

S. Rousse [a], S. Leroux [a], D. Gourichon [b], L. Calandreau [c] , I. David [a], A. Rau [d], S. Lagarrigue [e], T. Zerjal [d], F. Pitel [a], S.E. Eynard [a]

[a] *GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet-Tolosan,* France

[b] *PEAT, UE1295 - INRAE Centre Val de Loire, 37380, Nouzilly,* France

[c] *INRAE, CNRS, IFCE, Université de Tours, Centre Val de Loire UMR Physiologie de la Reproduction et des Comportements, 37380 Nouzilly,* France

[d] *GABI, Université Paris Saclay, INRAE, AgroParisTech, 78350 Jouy-en-Josas,* France

[e] *PEGASE, INRAE, Institut Agro, 35590 Saint Gilles,* France

Corresponding author:

Stacy Rousse. Email: stacy.rousse@inrae.fr

Sonia E. Eynard. Email: sonia.eynard@inrae.fr

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. The present study compares the phenotypic response for different phenotypes of two epilines of Japanese quails (*Coturnix japonica*), with similar genetic structure across three generations following an initial genistein ingestion in the ancestors' diet. Linear and linear mixed models were fitted to extract the fraction of variance allocated to multiple factors such as dam, family, sex, reproductive status

and epiline. The latter was found to be significantly associated with body weight and with heart, abdominal fat and testicle weight adjusted by 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 genistein treated offspring (epi+) and controls (epi-). These findings suggest a possible transgenerational effect of the initial diet disruption. In tissue weights, production and behavioural traits, the effect of the epiline accounted for smaller fractions of phenotypic variability and overall large residual variances were observed, underlining the complex nature of the analysed traits. This innovative experimental design allowed for a characterization of the impact of transgenerational effects on a variety of phenotypic traits, which will be useful for prioritizing traits for future genetic and epigenetic association studies.

Keywords

Epigenetic, Phenotype, Environmental modification, Transgenerational, *Coturnix japonica*

Implications

Nowadays, changes in the environment occur more frequently and livestock species can struggle to adapt to these rapid shifts. It is therefore important to understand the impact of the environment on animals. In this study, we evaluate how an initial modification in prior generations can also affect subsequent ones. By collecting different traits of interest (growth, tissues, production, and behaviour), we compare

the response to a change in the ancestors diet across three successive generations. Our findings highlight the importance of considering the potential multi-generational effects of changes to the environment. This could provide guidelines for better poultry management in order to ensure animal welfare while maintaining sustainable production yields.

Introduction

The evolution of farming practices as well as the increased frequency of environmental constraints due to climate change can be sources of stress for farm animals. Heat waves, variability of food accessibility and quality are examples of environmental changes livestock species must deal with. 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, non-genetic factors and the interaction between them. Among 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 not only to comprehend how animals adapt to changing environments but also to respond to 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. In this study, we align with this definition and 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. Transgenerational studies in farm animal are challenging due to the need to raise multiple generations and the large number of animals involved. To this end, quail is an ideal model for transgenerational 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. In addition, despite the predominance of chickens, Japanese quails (*Coturnix japonica*) were estimated to account for around 11% of the global share in poultry meat and egg production worldwide based on 2018 FAO data (Lukanov 2019). 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. However, to our knowledge, no study in birds has specifically examined the impact of a less intrusive environmental modification close to current rearing conditions, such as a shift in the dam diet on complex phenotypes of offspring. 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.

To investigate the existing link between the environment, the epigenome and the regulation of phenotypic expression at a transgenerational level, the present study 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). In human, genistein is known for its beneficial effects on health (reducing the risk of cancer, cardiovascular disease, diabetes, etc.), and today, its use in human medicine offers promising results for cancer treatment regarding its methylome regulation capacities (Rasheed et al., 2022; Casari et al., 2025). In certain regions of the world, a societal shift towards diets with lighter daily intake of animal proteins leads consumers to compensate with soy rich diets. However, numerous studies have demonstrated harmful effects of soy overconsumption on reproductive functions, in both sexes (Liu et al., 2020; Lozi et al., 2021; Rashid et al., 2022; Toktay et al., 2020) or cognitive functions (Svensson et al., 2021). The 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). Genistein supplementation is thus a promising candidate

method with potential to trigger phenotypic variation through changes on the methylome at a transgenerational level.

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 many poultry species, no direct quantification of the epigenetic influence on these traits has been reported. Regarding body weights, the genetic heritabilities described in the literature differ largely according to studies (Gaya et al., 2006, Moreira et al., 2019, Prince et al., 2020, Dadousis et al., 2021, Chomchuen et al., 2022, Wang et al., 2024) providing a compelling reason to investigate how much phenotypic variability is explained by epigenetic factors. The same conclusion can be drawn concerning behavioural traits, for which the environment, the emotional state and personalities account for large parts of the variabilities observed in individuals' response to stress (van Oers et al., 2003, Wright and Henriksen, 2020, Lormant et al., 2020) without quantifying the role of the epigenetic component. Overall, the objectives of this study are to 1) investigate the transgenerational transmission of the effects of an initial diet disruption across three generations of Japanese quails; 2) quantify the portion of phenotypic variability linked to epigenetic modifications and its evolution throughout generations; and 3) establish a starting point for further analyses using methylomes and genomes to establish on the transgenerational inheritance mechanisms underlying trait variability.

Material and methods

Quail mating design

All animals in the experiment are 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 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 for the three subsequent generations (G0, G1 and G2), inside a facility with water and feed *ad libitum* (Fig.1). From egg 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 placed in couples 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. 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.

Body weights (BW)

Body weights were measured at different developmental stages: one week, four weeks, seven weeks and slaughter 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. One-, four- and seven-week body weights were collected respectively at eight or nine days, thirty or thirty-two days and fifty or fifty-two days. The generation G-1 was slaughtered at thirty-one weeks (217 days), G0 and G2 at thirty weeks (210 or 211 days) and G1 at twenty-eight weeks (196 or 197 days). Slaughter was done through electronarcosis immediately following the recording of the final body measure.

Tissue weights (TW)

Several tissues were weighed: abdominal fat (for all generations), heart, liver (for G0 onwards), and testicle (for G1 onwards). Samples from those tissues were collected from a subset of the animals used to produce the next generation, immediately frozen in liquid nitrogen and stored for further analysis. 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 were recorded from the first day in the cage for 49 days (total egg number during the laying period, precocity). 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)

Before slaughter, the 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.

Statistical analysis : 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).

Statistical analysis : variance components estimation

To estimate the percentage of variance explained by influential factors such as the family, dam, sex and epiline, linear and linear mixed models were fitted to normalized trait variables.

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 phenotype, the optimal set of parameters was first determined with a linear model and 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+), reproductive status (0,1), family (origin from founders) and their pairwise interactions. Effects were iteratively added or deleted until the model with the lowest Akaike information criterion (AIC) was identified for each trait.

Linear mixed models (LMM)

In poultry, it is known that dams have a major effect on offspring growth and development through egg size and composition (Halbersleben DL and Mussekl FE, 1922; Widowski et al., 2022). Therefore, for some traits it is important to take into consideration the variability attributable to dam. To this end, a linear mixed model (LMM) with family, dam and interaction parameters as random effects using the R 'lme4' package (version 1.1-37) was fit as follows, where fixed effects corresponded to the ones obtained after LM selection :

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

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, $\boldsymbol{Z}$ is the associate matrix design for the random effects, α is the vector of random effects and ε is the residual.To select the best model for each trait (LM or LMM), ANOVA was used to determine whether the random effect of dam was significant. When it was the case, LMM was kept, otherwise we kept LM.

Cross Validations

After selecting the optimal models for each trait on the whole dataset, 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, which 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.squaredGLMM function from the R package 'MuMIn' (version 1.48.11).

Variance components

The proportion of variance explained by each factor for a given trait was estimated in one of two ways, according to whether the optimal model identified was a LM or a LMM. In the case of a LM, we performed an analysis of variance (ANOVA). 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 'car' (version 3.1-3) with Type II sum of squares.

In LMM, variance components for the fixed effects were computed using the post hoc calculation described by Hoffman and Schadt, 2016 where:

$$\hat{\sigma}^2_{\beta_j} = var(\boldsymbol{X}_j \widehat{\beta_j}),$$

where $\widehat{\beta_j}$ is the estimated effects for factor j with incidence matrix $\boldsymbol{X}_j$. The total variance is thus :

$$\hat{\sigma}^2_{Total} = \sum_j \hat{\sigma}^2_{\beta_j} + \sum_k \hat{\sigma}^2_{\alpha_k} + \sigma^2_{\varepsilon}$$

so that the proportion of variance explained by the jth fixed factor is:

$$PVE_j = \frac{\hat{\sigma}^2_{\beta_j}}{\hat{\sigma}^2_{Total}}$$

and the proportion of variance explained by the kth random factor is:

$$PVE_k = \frac{\hat{\sigma}^2_{\alpha_k}}{\hat{\sigma}^2_{Total}}.$$

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

Results

Impact of the epiline on phenotypes and evolution across generations

Significant differences between epilines were observed across several traits.

Body weights (BW)

For BW traits, females showed significant differences starting from generation G1 at all ages with epi+ quails systematically weighing more on average than controls. 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, which was not found to be significant. In G1, the difference between female epilines 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 (Fig. 3).

Tissue weights (TW)

For tissues, after adjustment on full body weight, significant weight differences between epilines were found in G1 for heart weight in both males and females, as well as in G2 males, although this latter result appears to be likely driven by the results of a single individual (Fig. 4).

Egg production (PROD)

For PROD traits, despite the absence of significant difference in laying rate, the difference between epilines seemed to increase on average in later generations, with epi+ quails showing signs of slightly better productivity, even though not significant than epi- individuals. For precocity and egg weight, results depended on the generation. Epi+ individuals were found to start laying eggs slightly later than controls in G1, although no difference was revealed in other generations. Egg weight was slightly heavier for epi+ in G0, but this tendency was erased in later generations (Fig. 5).

Behaviour at isolation (BHV)

Finally, in BHV traits, females of both epilines did not show any difference in behaviour for any of the traits, including in late generations. Epi- males in G2 exhibited a significantly stronger agitation (frequency at centre and at periphery higher on average) than their epi+ counterparts, most probably due to the exploratory behaviour of a few individuals only, but this observation did not translate into higher total distance or velocity. In G1, epi- quails were found to spend significantly more time at the centre of the arena though this tendency was not observed in other generations (Fig. 6). Only the total distance, the frequency at periphery and the duration at centre were considered in the next steps.

Phenotypic variance explained by parameters: evolution across generations

Effect of the epiline

Model fitting revealed that epiline as well as other parameters such as dam, sex, family and reproductive status explained a significant portion of the variability observed in the traits considered. For BW, epiline was found significant at all ages, from G1 onwards. In addition, it was found to account for more variability in later generations, with an increase in the percentage of variance explained going respectively from 0 (G0) to 5.5% (G2) at one week of age, 1.3 (G0) to 9.4% (G2) at four weeks, 0.5 (G0) to 6.7% (G2) at seven weeks and from 0.02 (G0) to 2.7% (G2) at slaughter. A similar pattern across generations was observed for other traits, including total weight adjusted abdominal fat (0.06% in G0 to 0.8% in G2) and testicle (0 to 2.6% in G2) and frequency at the periphery (0% in G0 to 0.9% in G2). Other phenotypes like egg weight and duration at centre behaved differently, with the portion of variation explained by epiline decreasing between G0 and G2 (resp. 2.4%

in G0 to 0.5% in G2 for egg weight and 0.8% to 0% in G2 for the duration at centre). Lastly, adjusted heart weight, precocity and total distance showed a higher percentage of variance explained by the epiline in G2 compared to G0, but lower than in G1. The laying rate and liver weight did not show a significant part of variability related to epiline (Table 2). In a fair share of traits (BW, abdominal fat weight, testicle weight, egg weight, precocity, total distance), a significant interaction parameter involving the epiline was found, adding to the overall effect of this factor (Fig. 7). Details on the estimation of the percentage of phenotypic variance related to the interaction with epiline (among other parameters) are available in Supplementary Table S2.

Effect of other factors

Concerning other parameters, expectedly the dam effect had a stronger influence on BW variability in younger individuals, and was influential in two of the three PROD traits (Fig. 7). In G0, it accounted for 7.7% of the BW at one week of age and decreased to 3.9% at slaughter (Fig. 7A). This pattern intensified in G1 and G2, with overall higher effect of the dam (from 16.1% at one week to 3.1% at slaughter in G1 and resp. 37.2% to 5.2% in G2). In PROD traits, the effect of the dam was somewhat similar for egg weight with an increase in G2 compared to G1 (25.9% compared to 19.5%) but no variation was observed for precocity between G0 and G2, where it accounted for almost a third of the total variance (Fig. 7B).

Contrary to the increase in dam effect across generations for BW, the share of variance explained by the family declined, starting from 42.3% at one week in G0 to 16.8% in G1 and decreasing again to 3.2% in G2 (Fig. 7A). The family effect was also stronger in young animals than adults. On the other hand, the influence of family slightly strengthened across generations for adjusted abdominal fat, doubling in G2

from explaining 6% in G0. In other phenotypes like BHV traits, the family was found significant for total distance at all generations and for the frequency at periphery in G0 and G1, but with no striking change of its influence. For these traits, family was found to explain between 10 to 15% of the overall variance (Fig. 7B).

As expected, sex had relevant effects on the variability of adult quail traits. In BW, the proportion of variability explained by sex increased with age, from nearly 0% to 1% at one week of age to over 50% at slaughter. (Fig. 7A). In TW and BHV, the amount of variance explained by sex was trait dependent, yet variable across generations, with the exception of duration at centre where the model struggled to capture the variability. It was strongest for liver weight where it ranged from 41% to 70%, lighter for heart weight (from 7.7 to 29%) and total distance (8 to 20%); the sex component was least important for abdominal fat (1.3 to 8.9%) and frequency at periphery (5 to 10%) (Fig. 7B).

The reproductive status of quails was found to be significantly involved in two traits (testicle weight in G1 and in laying rate in G0 and G1), where it explained between 3 to 9% of the variability.

Cross validations

The predictive performance of the models, evaluated using cross validations, varied considerably across traits and generations. Traits related to BW, such as BW at slaughter, consistently exhibited the highest prediction accuracy across all generations ($R^2 \geq 0.79$, Corr ≥ 0.86), highlighting good model performance for growth-related traits. In contrast, behavioural traits like the duration at centre showed the lowest coefficients of determination and predictive values ($R^2 \leq 0.04$, Corr ≤ 0.16), suggesting limited predictive power of our models for these traits; these results are in line with the large residual variance components estimated for behavioural traits. For

PROD traits, adjusted egg weight had moderate R^2 values in G0 (0.56) but decreased in G1 (0.23), recovering somewhat in G2 (0.37); the corresponding correlation values followed a similar trend. Interestingly, the models struggled to fit the laying rate phenotype with an extremely low R^2 (0.06) in G0 to a moderate value in G2 (0.32) and, overall low correlation values, suggesting the limited capacity of the models for this trait. Among TW traits, liver weight demonstrated high predictive power in G0 and G2 (R^2= 0.73 and 0.68, Corr = 0.76 and 0.82, respectively). These results underline the differential predictability of traits, with body composition and weight traits generally yielding higher R^2 and correlation values compared to behaviour or some production-related traits (Table 3).

Discussion

The aim of this study was to analyse phenotypic variations across three generations of quails following supplementation with genistein, an endocrine disruptor.

Genistein ingestion is associated with different phenotypic responses across generations

In both sexes, body weight (BW) was increased 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 contrast, we did not observe such sex-specific interactions in the present study, regardless of the generation or age considered. On the other hand,

the results obtained concur with other studies concerning the significant impact of genistein on BW, even though 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 individual.

Additionally, 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, even though epiline was systematically kept as a factor in the model, explaining a marginal yet significant portion of the precocity and the egg weight variances. Likewise, abdominal fat weight and behaviour (BHV) traits did not demonstrate the previously reported significant differences between epilines. Here, they only explained a limited fraction of the variability depending on the generation considered. 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). These different sensitivities may explain to a certain extent the contrasts observed in potential transmission

patterns, with more pronounced effect of the epiline in BW traits in later generations compared to PROD or BHV traits.

Missing variability highlights the complexity of phenotypes

The proportion of phenotypic variability explained by epigenetic (epiline) or genetic (dam, family) factors displayed a clear trend with age, decreasing over the lifetime (with the exception of week 4 in G1). This may reflect the growing influence of other factors on BW variability, particularly the sex of the individual. Regardless, residual variance was substantial across all traits. 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 traits. A possible explanation resides in 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. Regarding PROD traits, residual variance can be overestimated due to the remaining skewness of the traits, even after applying a transformation to normalize data. Heritabilities reported in the literature for these phenotypes are thus impacted and can be very low, even in selected lines of laying hens (Wolc et al., 2012). Additionally, limited sample sizes in each generation can complicate the accurate estimation of genetic parameters and phenotypic scores, as revealed by the correlation between observed and predicted values. Another example on a New Zealand bird species 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 multi and transgenerational transmission of genistein effect

The availability of phenotypic records for all individuals of the design highlighted a progressive increase of the epiline effect from G0 to G2 in a handful of traits (TW, BW, precocity). Interestingly, this phenomenon was strongly emphasized in BW traits at all ages, where the percentage of variance explained by the epiline largely amplified from G0 to G2. Adjusted liver, abdominal fat and testicle weights displayed a similar trend as BW, despite the overall modest percentage of variability associated with the epiline for those phenotypes. The progressive impact of the epiline across generations might be explained by 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, an increasing environmental influence across generations 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 (submitted). Indeed, the authors identified a significant difference between expected and estimated sire and dams path coefficients, marking a likely environmental effect on BW traits from the same dataset. Their result supports the possible 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 environmental exposure during development can induce persistent differences between epilines, particularly on BW. The increasing proportion of phenotypic variance explained by the epiline across generations suggests the potential involvement of non-genetic inheritance mechanisms, although the contribution of genetic drift cannot be excluded despite the controlled mating design and the absence of progeny selection (Supplementary Figure S1). To better disentangle these effects, future studies could focus on integrating genome-wide epigenetic profiling (e.g., DNA methylation) across generations. For the traits showing epiline effect, running EWAS and metQTL analyses to identify genomic regions where epigenetic marks govern phenotypic changes or where genetic variation is driving epigenetic changes will help decipher the variance due to genetic and epigenetic mechanisms.

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.

Author ORCIDs

S.Rousse: https://orcid.org/0009-0007-2894-9356
S.Leroux: https://orcid.org/0000-0003-2840-5109
D.Gourichon: https://orcid.org/0000-0002-4631-0149
L.Calandreau: https://orcid.org/0000-0002-7535-5733
I.David: https://orcid.org/0000-0002-2514-6693
A.Rau: https://orcid.org/0000-0001-6469-488X
S.Lagarrigue: https://orcid.org/0000-0002-4887-7245
T.Zerjal: https://orcid.org/0000-0002-0666-5420
F.Pitel: https://orcid.org/0000-0002-1477-7633
S.Eynard: https://orcid.org/0000-0002-8609-5869

Declaration of interest

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

Acknowledgements

The authors would like to thank Mary Ann Ottinger and Catherine Bennetau-Pelissero for their advice and help concerning genistein. Many thanks to all the collaborators and partners, in particular the colleagues from PEAT, especially David Gourichon, Sandrine Rivière and Michael Troquet who carefully reared the quails and kindly helped for all experiments. Finally, special thanks to the Genobioinfo platform for data management.

Financial support statement

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N°101000236 (GEroNIMO). This project is part of EuroFAANG (https://eurofaang.eu). Stacy Rousse is co-funded by the INRAE Animal Genetics Division and the French Occitanie Region.

References

Bartoń, K., 2025. MuMIn: Multi-Model Inference. doi:10.32614/CRAN.package.MuMIn <https://doi.org/10.32614/CRAN.package.MuMIn>, R package version 1.48.11, <https://CRAN.R-project.org/package=MuMIn>.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1),1-48. doi:10.18637/jss.v067.i01.

Béziers, P., San-Jose, L.M., Almasi, B., Jenni, L., Roulin, A., 2019. Baseline and stress-induced corticosterone levels are heritable and genetically correlated in a barn owl population. Heredity 123, 337–348. https://doi.org/10.1038/s41437-019-0203-5

Brulport, A., Le Corre, L., Maquart, G., Barbet, V., Dastugue, A., Severin, I., Vaiman, D., Chagnon, M.-C., 2021. Multigenerational study of the obesogen effects of bisphenol S after a perinatal exposure in C57BL6/J mice fed a high fat diet. Environ. Pollut. 270, 116243. https://doi.org/10.1016/j.envpol.2020.116243

Brun, J.-M., Bernadet, M.-D., Cornuez, A., Leroux, S., Bodin, L., Basso, B., Davail, S., Jaglin, M., Lessire, M., Martin, X., Sellier, N., Morisson, M., Pitel, F., 2015. Influence of grand-mother diet on offspring performances through the male line in Muscovy duck. BMC Genet. 16, 145. https://doi.org/10.1186/s12863-015-0303-z

Casari, G., Romaldi, B., Scirè, A., Minnelli, C., Marzioni, D., Ferretti, G., Armeni, T., 2025. Epigenetic Properties of Compounds Contained in Functional Foods Against Cancer. Biomolecules 15, 15. https://doi.org/10.3390/biom15010015

Charrier, M., Lumineau, S., George, I., Meurisse, M., Georgelin, M., Palme, R., Angelier, F., Coustham, V., Nicolle, C., Bertin, A., Darmaillacq, A.-S., Dickel, L., Guémené, D., Calandreau, L., Houdelier, C., 2024. Maternal stress effects across generations in a precocial bird. R. Soc. Open Sci. 11, 231826. https://doi.org/10.1098/rsos.231826

Chomchuen, K., Tuntiyasawasdikul, V., Chankitisakul, V., Boonkum, W., 2022. Genetic Evaluation of Body Weights and Egg Production Traits Using a Multi-Trait Animal Model and Selection Index in Thai Native Synthetic Chickens (Kaimook e-san2). Anim. Open Access J. MDPI 12, 335. https://doi.org/10.3390/ani12030335

Dadousis, C., Somavilla, A., Ilska, J.J., Johnsson, M., Batista, L., Mellanby, R.J., Headon, D., Gottardo, P., Whalen, A., Wilson, D., Dunn, I.C., Gorjanc, G., Kranis, A., Hickey, J.M., 2021. A genome-wide association analysis for body weight at 35 days measured on 137,343 broiler chickens. Genet. Sel. Evol. 53, 70. https://doi.org/10.1186/s12711-021-00663-w

Dolinoy, D.C., Weidman, J.R., Waterland, R.A., Jirtle, R.L., 2006. Maternal Genistein Alters Coat Color and Protects Avy Mouse Offspring from Obesity by Modifying the Fetal Epigenome. Environ. Health Perspect. 114, 567–572. https://doi.org/10.1289/ehp.8700

Duncan, E.J., Gluckman, P.D., Dearden, P.K., 2014. Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? J. Exp. Zoolog. B Mol. Dev. Evol. 322, 208–220. https://doi.org/10.1002/jez.b.22571

Falconer, D.S. and Mackay, T.F.C. (1996). Introduction to quantitative genetics. 4 . Ed. Harlow,UK, Longman Group, 464 p. ISBN 978-0-582-24302-6

Fonseca, P.A.S., Suárez-Vega, A., Pelayo, R., Marina, H., Alonso-García, M., Gutiérrez-Gil, B., Arranz, J.-J., 2023. Intergenerational impact of dietary protein restriction in dairy ewes on epigenetic marks in the perirenal fat of their suckling lambs. Sci. Rep. 13, 4351. https://doi.org/10.1038/s41598-023-31546-3

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. <https://www.john-fox.ca/Companion/>.

Frésard, L., Morisson, M., Brun, J.-M., Collin, A., Pain, B., Minvielle, F., Pitel, F., 2013. Epigenetics and phenotypic variability: some interesting insights from birds. Genet. Sel. Evol. 45, 16. https://doi.org/10.1186/1297-9686-45-16

Gaya, L.G., Ferraz, J.B.S., Rezende, F.M., Mourão, G.B., Mattos, E.C., Eler, J.P., Michelan Filho, T., 2006. Heritability and Genetic Correlation Estimates for Performance and Carcass and Body Composition Traits in a Male Broiler Line. Poult. Sci. 85, 837–843. https://doi.org/10.1093/ps/85.5.837

Guerrero-Bosagna, C., Morisson, M., Liaubet, L., Rodenburg, T.B., de Haas, E.N., Košťál, Ľ., Pitel, F., 2018. Transgenerational epigenetic inheritance in birds. Environ. Epigenetics 4, dvy008. https://doi.org/10.1093/eep/dvy008

Halbersleben DL and Mussekl FE. Relationship of egg weight to chickweight at hatching. Poult Sci 1: 143–144, 1922

Hoffman, G.E., Schadt, E.E., 2016. variancePartition: interpreting drivers of variation in complex gene expression studies. BMC Bioinformatics 17, 483. https://doi.org/10.1186/s12859-016-1323-z

Hospital, F., Chevalet, C., 1996. Interactions of selection, linkage and drift in the dynamics of polygenic characters. Genet. Res. 67, 77–87. https://doi.org/10.1017/s0016672300033498

Hu, Y., Feng, Y., Ding, Z., Lv, L., Sui, Y., Sun, Q., Abobaker, H., Cai, D., Zhao, R., 2020. Maternal betaine supplementation decreases hepatic cholesterol deposition in chicken offspring with epigenetic modulation of SREBP2 and CYP7A1 genes. Poult. Sci. 99, 3111–3120. https://doi.org/10.1016/j.psj.2019.12.058

Kassambara, A., 2023. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. doi:10.32614/CRAN.package.rstatix <https://doi.org/10.32614/CRAN.package.rstatix>, R package version 0.7.2, <https://CRAN.R-project.org/package=rstatix>.

Kiefer, H., Sellem, E., Bonnet-Garnier, A., Pannetier, M., Costes, V., Schibler, L., Jammes, H., 2021. The epigenome of male germ cells and the programming of phenotypes in cattle. Anim. Front. 11, 28–38. https://doi.org/10.1093/af/vfab062

Komsta, L., Novomestky, F., 2022. moments: Moments, Cumulants, Skewness, Kurtosis and Related Tests. doi:10.32614/CRAN.package.moments <https://doi.org/10.32614/CRAN.package.moments>, R package version 0.14.1, <https://CRAN.R-project.org/package=moments>.

Leroux, S., Gourichon, D., Leterrier, C., Labrune, Y., Coustham, V., Rivière, S., Zerjal, T., Coville, J.-L., Morisson, M., Minvielle, F., Pitel, F., 2017. Embryonic environment and transgenerational effects in quail. Genet. Sel. Evol. 49, 14. https://doi.org/10.1186/s12711-017-0292-7

Lin, F., Wu, J., Abdelnabi, M.A., Ottinger, M.A., Giusti, M.M., 2004. Effects of Dose and Glycosylation on the Transfer of Genistein into the Eggs of the Japanese Quail (Coturnix japonica). J. Agric. Food Chem. 52, 2397–2403. https://doi.org/10.1021/jf034921f

Liu, X., Li, F., Xie, J., Huang, D., Xie, M., 2020. Fetal and neonatal genistein exposure aggravates to interfere with ovarian follicle development of obese female mice induced by high-fat diet. Food Chem. Toxicol. 135, 110982. https://doi.org/10.1016/j.fct.2019.110982

Lormant, F., Ferreira, V.H.B., Meurisse, M., Lemarchand, J., Constantin, P., Morisse, M., Cornilleau, F., Parias, C., Chaillou, E., Bertin, A., Lansade, L., Leterrier, C., Lévy, F., Calandreau, L., 2020. Emotionality modulates the impact of chronic stress on memory and neurogenesis in birds. Sci. Rep. 10, 14620. https://doi.org/10.1038/s41598-020-71680-w

Lozi, A.A., Pinto da Matta, S.L., Sarandy, M.M., Silveira Alves de Melo, F.C., Araujo, D.C., Novaes, R.D., Gonçalves, R.V., 2021. Relevance of the Isoflavone Absorption and Testicular Function: A Systematic Review of Preclinical Evidence. Evid. Based Complement. Alternat. Med. 2021, 8853172. https://doi.org/10.1155/2021/8853172

Lukanov, H., 2019. Domestic quail (Coturnix japonica domestica), is there such farm animal? Worlds Poult. Sci. J. 75, 547–558. https://doi.org/10.1017/S0043933919000631

Lv, Z., Fan, H., Zhang, B., Xing, K., Guo, Y., 2018. Dietary genistein supplementation for breeders and their offspring improves the growth performance and immune function of broilers. Sci. Rep. 8, 5161. https://doi.org/10.1038/s41598-018-23530-z

Mills, A.D., Faure, J.-M., 1991. Divergent selection for duration of tonic immobility and social reinstatement behavior in Japanese quail (Coturnix coturnix japonica) chicks. J. Comp. Psychol. 105, 25–38. https://doi.org/10.1037/0735-7036.105.1.25

Moreira, G.C.M., Poleti, M.D., Pértille, F., Boschiero, C., Cesar, A.S.M., Godoy, T.F., Ledur, M.C., Reecy, J.M., Garrick, D.J., Coutinho, L.L., 2019. Unraveling genomic associations with feed efficiency and body weight traits in chickens through an integrative approach. BMC Genet. 20, 83. https://doi.org/10.1186/s12863-019-0783-3

Morris, K.M., Hindle, M.M., Boitard, S., Burt, D.W., Danner, A.F., Eory, L., Forrest, H.L., Gourichon, D., Gros, J., Hillier, L.W., Jaffredo, T., Khoury, H., Lansford, R., Leterrier, C., Loudon, A., Mason, A.S., Meddle, S.L., Minvielle, F., Minx, P., Pitel, F., Seiler, J.P., Shimmura, T., Tomlinson, C., Vignal, A., Webster, R.G., Yoshimura, T., Warren, W.C., Smith, J., 2020. The quail genome: insights into social behaviour, seasonal biology and infectious disease response. BMC Biol. 18, 14. https://doi.org/10.1186/s12915-020-0743-4

Navarro-Guillén, C., Huesa-Cerdán, R., Hidalgo-Pérez, J.A., Simó-Mirabet, P., Rodríguez-Viera, L., Martos-Sitcha, J.A., Martínez-Rodríguez, G., Perera, E., 2025. One□carbon nutrients and genistein as nutritional programming effectors in juvenile gilthead seabream (Sparus aurata): Contrasting effects on phenotypic traits. Aquaculture 598, 742063. https://doi.org/10.1016/j.aquaculture.2024.742063

Onderci, M., Sahin, K., Sahin, N., Gursu, M.F., Doerge, D., Sarkar, F.H., Kucuk, O., 2004. The effect of genistein supplementation on performance and antioxidant status of Japanese quail under heat stress. Arch. Anim. Nutr. 58, 463–471. https://doi.org/10.1080/00039420400020017

Prince, L.L.L., Rajaravindra, K.S., Rajkumar, U., Reddy, B.L.N., Paswan, C., Haunshi, S., Chatterjee, R.N., 2020. Genetic analysis of growth and egg production traits in synthetic colored broiler female line using animal model. Trop. Anim. Health Prod. 52, 3153–3163. https://doi.org/10.1007/s11250-020-02340-4

R Core Team (2025). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Rasheed, S., Rehman, K., Shahid, M., Suhail, S., Akash, M.S.H., 2022. Therapeutic potentials of genistein: New insights and perspectives. J. Food Biochem. 46, e14228. https://doi.org/10.1111/jfbc.14228

Rashid, R., Kumari, A., Chattopadhyay, N., Jha, R., Rajender, S., 2022. Genistein lowers fertility with pronounced effect in males: Meta-analyses on pre-clinical studies. Andrologia 54, e14511. https://doi.org/10.1111/and.14511

Rockwood, S., Mason, D., Lord, R., Lamar, P., Prozialeck, W., Al-Nakkash, L., 2019. Genistein diet improves body weight, serum glucose and triglyceride levels in both male and female ob/ob mice. Diabetes Metab. Syndr. Obes. Targets Ther. 12, 2011–2021. https://doi.org/10.2147/DMSO.S216312

Rutschmann, A., de Villemereuil, P., Brekke, P., Ewen, J.G., Anderson, N., Santure, A.W., 2020. Consequences of space sharing on individual phenotypes in the New Zealand hihi. Evol. Ecol. 34, 821–839. https://doi.org/10.1007/s10682-020-10063-z

Skinner, M.K., 2008. What is an epigenetic transgenerational phenotype? Reprod. Toxicol. 25, 2–6. https://doi.org/10.1016/j.reprotox.2007.09.001

Svensson, T., Sawada, N., Mimura, M., Nozaki, S., Shikimoto, R., Tsugane, S., 2023. Midlife Intakes of the Isoflavone Genistein and Soy and the Risk of Late-life Cognitive

Impairment: The JPHC Saku Mental Health Study. J. Epidemiol. 33, 342–349. https://doi.org/10.2188/jea.JE20210199

Toktay, E., Selli, J., Gurbuz, M.A., Tastan, T.B., Ugan, R.A., Un, H., Halici, Z., 2020. Effects of soy isoflavonoids (genistein and daidzein) on endometrial receptivity. Iran. J. Basic Med. Sci. 23, 1603–1609. https://doi.org/10.22038/ijbms.2020.48294.11089

van Oers, K., Drent, P.J., de Goede, P., van Noordwijk, A.J., 2004. Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. Proc. R. Soc. Lond. B Biol. Sci. 271, 65–73. https://doi.org/10.1098/rspb.2003.2518

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Vitorino Carvalho, A., Hennequet-Antier, C., Rouger, R., Delaveau, J., Bordeau, T., Crochet, S., Couroussé, N., Pitel, F., Collin, A., Coustham, V., 2023. Thermal conditioning of quail embryos has transgenerational and reversible long-term effects. J. Anim. Sci. Biotechnol. 14, 124. https://doi.org/10.1186/s40104-023-00924-2

Wang, J., Liu, J., Lei, Q., Liu, Z., Han, H., Zhang, S., Qi, C., Liu, W., Li, D., Li, F., Cao, D., Zhou, Y., 2024. Elucidation of the genetic determination of body weight and size in Chinese local chicken breeds by large-scale genomic analyses. BMC Genomics 25, 296. https://doi.org/10.1186/s12864-024-10185-6

Widowski, T.M., Cooley, L., Hendriksen, S., Peixoto, M.R.L.V., 2022. Maternal age and maternal environment affect egg composition, yolk testosterone, offspring growth and behaviour in laying hens. Sci. Rep. 12, 1828. https://doi.org/10.1038/s41598-022-05491-6

Wolc, A., Arango, J., Settar, P., O'Sullivan, N.P., Olori, V.E., White, I.M.S., Hill, W.G., Dekkers, J.C.M., 2012. Genetic parameters of egg defects and egg quality in layer chickens. Poult. Sci. 91, 1292–1298. https://doi.org/10.3382/ps.2011-02130

Wright, D., Henriksen, R., 2020. Advances in understanding the genetics of poultry behaviour. In Understanding the behaviour and improving the welfare of chickens. Burleigh Dodds Series In Agriculture Science Number 91. (ed. Prof. Nicol, C. Royal

Veterinary College – University of London, UK). Burleigh Dodds Science Publishing. DOI 10.19103/AS.2020.0078. pp 3-217

Wu, P., Wang, Junge, Ji, X., Chai, J., Chen, L., Zhang, T., Long, X., Tu, Z., Chen, S., Zhang, Lijuan, Wang, K., Zhang, Liang, Guo, Z., Wang, Jinyong, 2024. Maternal Hypermethylated Genes Contribute to Intrauterine Growth Retardation of Piglets in Rongchang Pigs. Int. J. Mol. Sci. 25, 6462. https://doi.org/10.3390/ijms25126462

Xiang, J., Mlambo, R., Dube, P., Machona, O., Shaw, I., Seid, Y., He, Y., Luo, M., Hong, T., He, B., Zhou, W., Tan, S., 2023. The obesogenic side of Genistein. Front. Endocrinol. 14. https://doi.org/10.3389/fendo.2023.1308341

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 phenotypic variance explained by the epiline in each generation.

Type	Trait	G0	G1	G2
BW	1 week	0	2.846	5.487
			[2.139;3.552]	[4.688;6.285]
	4 weeks	1.304	5.329	9.375
		[0.806;1.802]	[4.232;6.425]	[7.779;10.97]
	7 weeks	0.471	3.624	6.718
		[0.204;0.738]	[2.733;4.515]	[5.544;7.892]
	slaughter	0.023	0.792	2.693
		[-0.01;0.055]	[0.565;1.019]	[2.267;3.118]
TW	abdo. fat[1]	0.063	0.168	0.815
		[-0.021;0.146]	[-0.018;0.354]	[0.398;1.233]
	heart	0.21	4.12	0.42
		[-0.099;0.519]	[3.231;5.009]	[0.113;0.727]
	liver	0	0	0.018
				[-0.004;0.039]
	testicle	-	0.136	2.622
			[-0.033;0.305]	[1.453;3.79]
PROD	laying rate	0	0	0
	precocity	0.267	3.205	0.166
		[-0.077;0.611]	[2.152;4.259]	[-0.083;0.415]
	egg weight	2.438	0.38	0.514
		[1.464;3.412]	[-0.003;0.764]	[0.016;1.012]
BHV	total distance	0	0.228	0.079
			[0.024;0.432]	[-0.028;0.186]
	freq. at	0	0.598	0.958
	periphery[2]		[0.22;0.977]	[0.51;1.406]
	duration at	0.792	0.227	0
	centre	[0.384;1.2]	[-0.016;0.47]	

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

[1] Abdominal fat weight

[2] Frequency at periphery

Table 3

Percentage of phenotypic variance explained by the epiline in each generation.

Type	Trait	Generation	R^2 av	R^2 sd	Corr av	Corr sd
BW	1 week	G0	0.48	0.03	0.63	0.06
		G1	0.37	0.03	0.48	0.08
		G2	0.47	0.02	0.59	0.06
	4 weeks	G0	0.4	0.03	0.54	0.07
		G1	0.36	0.03	0.47	0.07
		G2	0.53	0.02	0.65	0.05
	7 weeks	G0	0.48	0.03	0.59	0.07
		G1	0.48	0.03	0.6	0.06
		G2	0.57	0.02	0.68	0.05
	slaughter	G0	0.81	0.01	0.88	0.02
		G1	0.79	0.01	0.88	0.02
		G2	0.79	0.02	0.86	0.03
TW	abdo. fat	G0	0.49	0.04	0.47	0.09
		G1	0.48	0.02	0.52	0.07
		G2	0.35	0.02	0.34	0.09
	heart	G0	0.11	0.04	0.25	0.22
		G1	0.43	0.02	0.6	0.06
		G2	0.28	0.02	0.34	0.09
	liver	G0	0.73	0.02	0.76	0.07
		G1	0.45	0.02	0.66	0.07
		G2	0.68	0.01	0.82	0.03
	testicle	G1	0.31	0.06	0.35	0.11
		G2	0.42	0.05	0.38	0.12
PROD	laying rate	G0	0.06	0.01	0.25	0.09
		G1	0.29	0.03	0.23	0.12
		G2	0.32	0.03	0.2	0.13
	precocity	G0	0.37	0.07	0.36	0.15
		G1	0.03	0.01	0.16	0.15
		G2	0.35	0.09	0.33	0.13
	egg weight	G0	0.56	0.03	0.48	0.11
		G1	0.23	0.07	0.18	0.15
		G2	0.37	0.05	0.35	0.13
BHV	total	G0	0.32	0.02	0.46	0.07
		G1	0.32	0.02	0.43	0.08
	distance	G2	0.32	0.02	0.26	0.1
	freq. at	G0	0.21	0.02	0.29	0.09
		G1	0.25	0.02	0.36	0.09
	periphery	G2	0.06	0.01	0.22	0.1
	duration at	G0	0.04	0.01	0.16	0.09
		G1	0.04	0.01	0.11	0.12
	centre	G2	0.01	0	0.14	0.03

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

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

Figure captions

Fig. 1. Quail mirror mating design. Genistein capsules were administered to females in G-1 as 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.

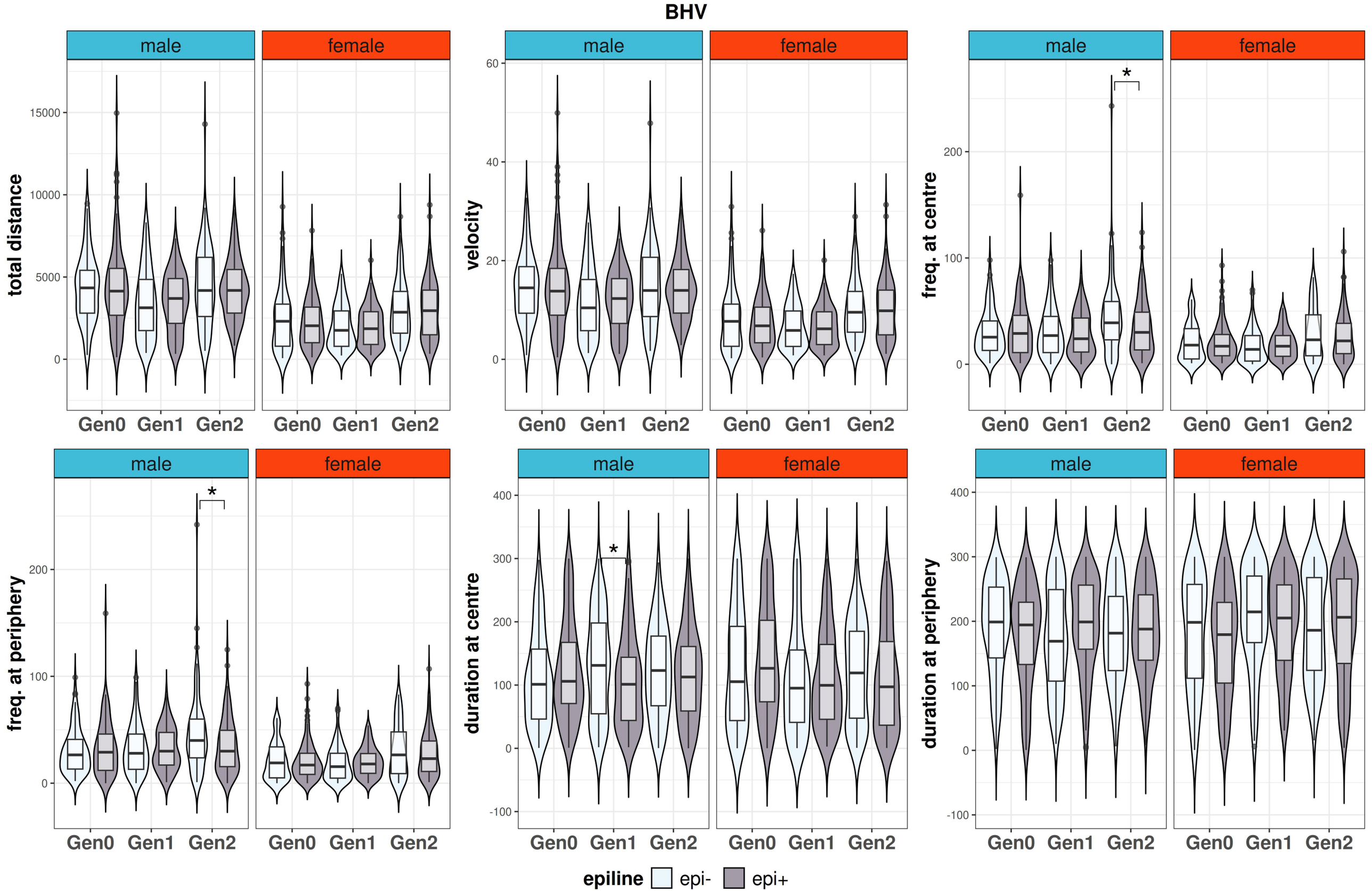
Fig. 3. Average body weight differences between epilines throughout quails' lifetime across generations.

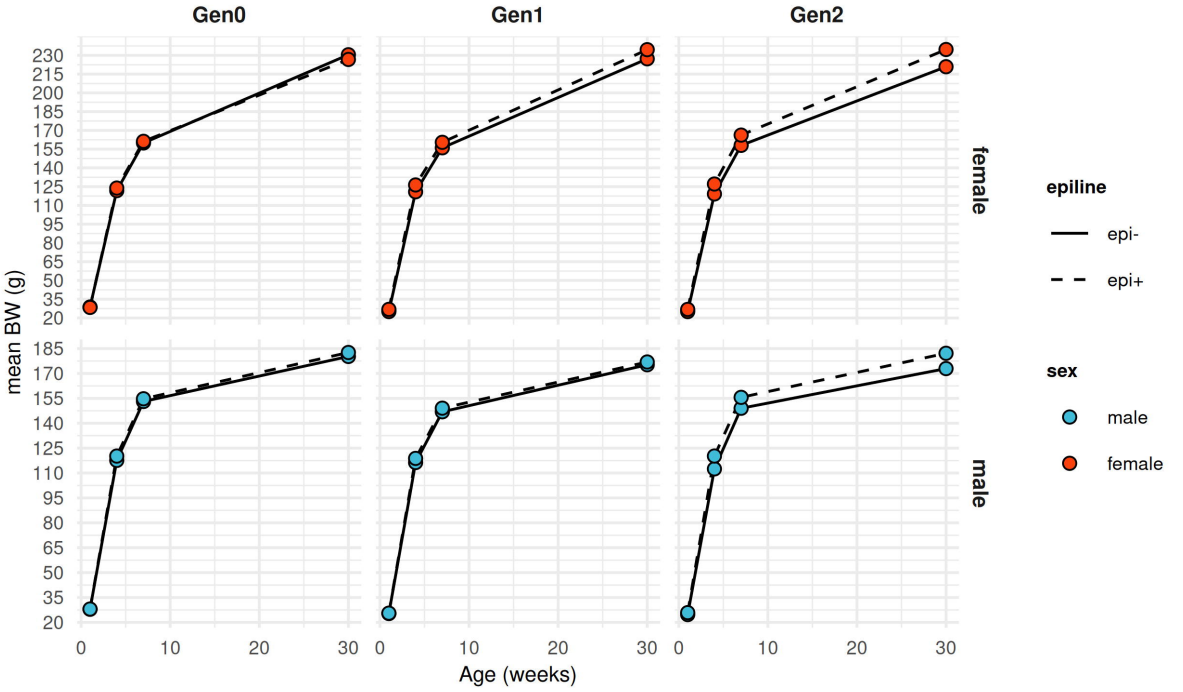
Fig. 4. Analysis of variance on adjusted tissue weights (heart, liver, abdominal fat and testicle) between epilines. The study was conducted by sex for abdominal fat, heart and liver weights and by generation for all tissues. All weights were adjusted by the body weight at slaughter. The asterisk indicates the level of significance of the Tukey-HSD test results.

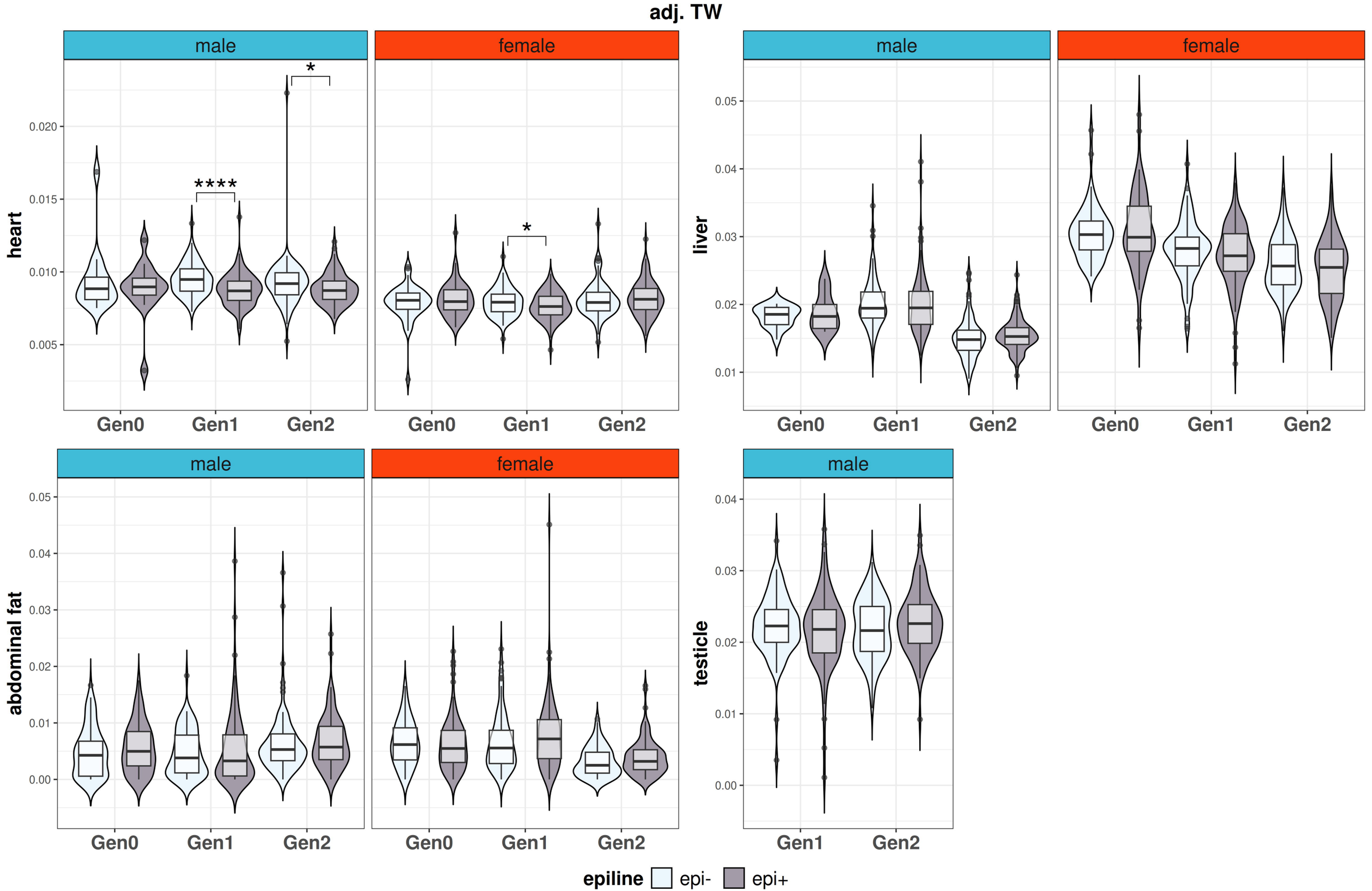
Fig. 5. Analysis of variance on production traits (laying rate, precocity and egg weight) between epilines. The study was conducted by generation. The red dotted line in the distribution of laying rate corresponds to the mean value of each epiline. Egg weight was corrected by the body weight at slaughter. The asterisk indicates the level of significance of the Tukey-HSD test results.

Fig. 6. Analysis of variance on behavioural traits (total distance, velocity, frequency at centre, frequency at periphery, duration at centre and duration at periphery) between epilines. The study was conducted by sex and generation. The asterisk indicates the level of significance of the Tukey-HSD test results.

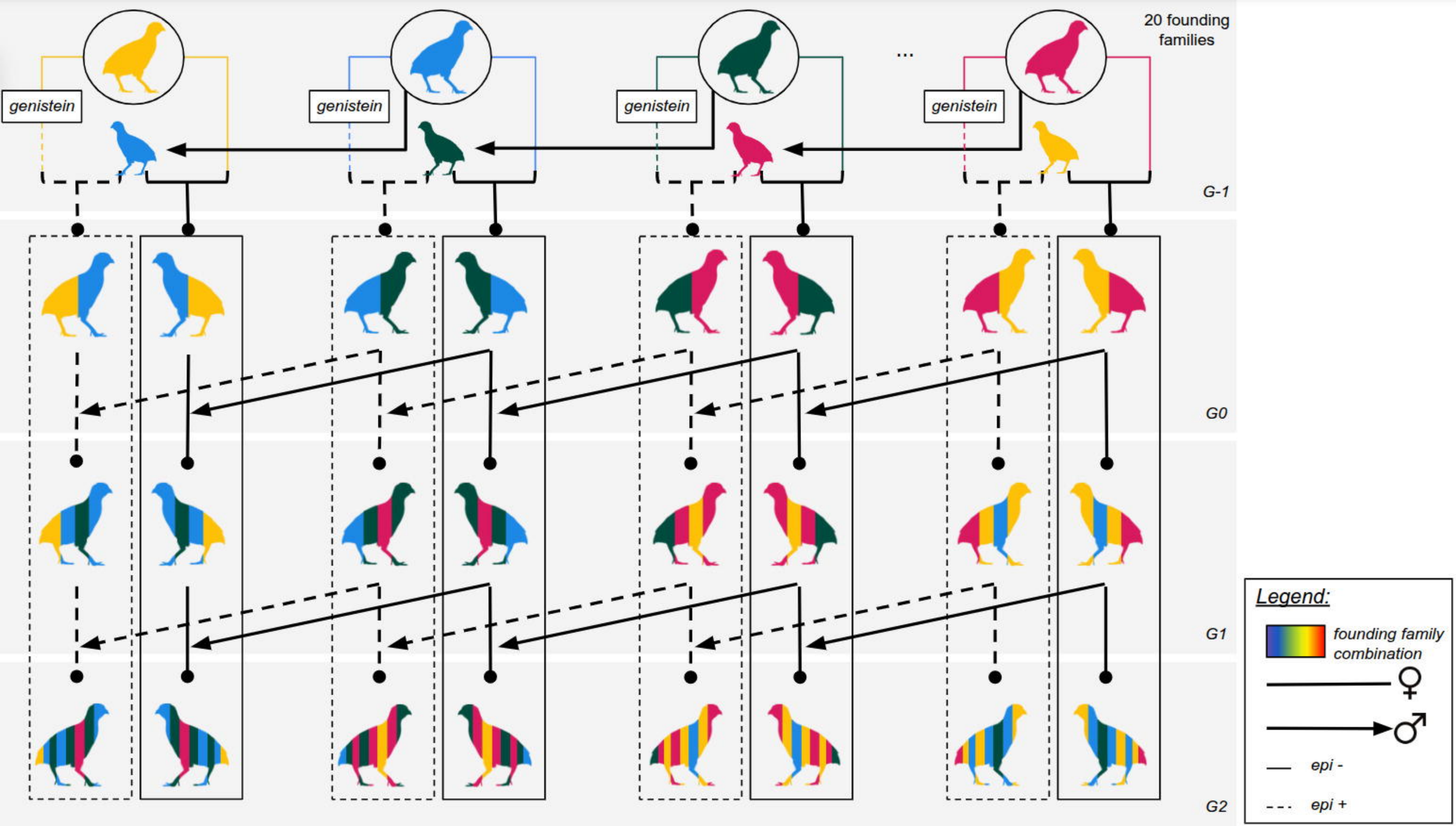
Fig. 7. Phenotypic variance partition between all factors (dam, sex, family, epiline, reproductive status and interaction) per generation. The interaction is split into 2 factors: the interaction parameter involving epiline and the interaction parameter involving other factors. **A**: Body weights at one, four, seven and thirty (slaughter) weeks of age in G0, G1 and G2; **B**: Tissue weights (abdominal fat, liver, heart and testicle weights), Production traits (laying rate, precocity, and egg weight) and Behavioural traits (total distance, duration at centre and frequency at the periphery) in G0, G1 and G2.

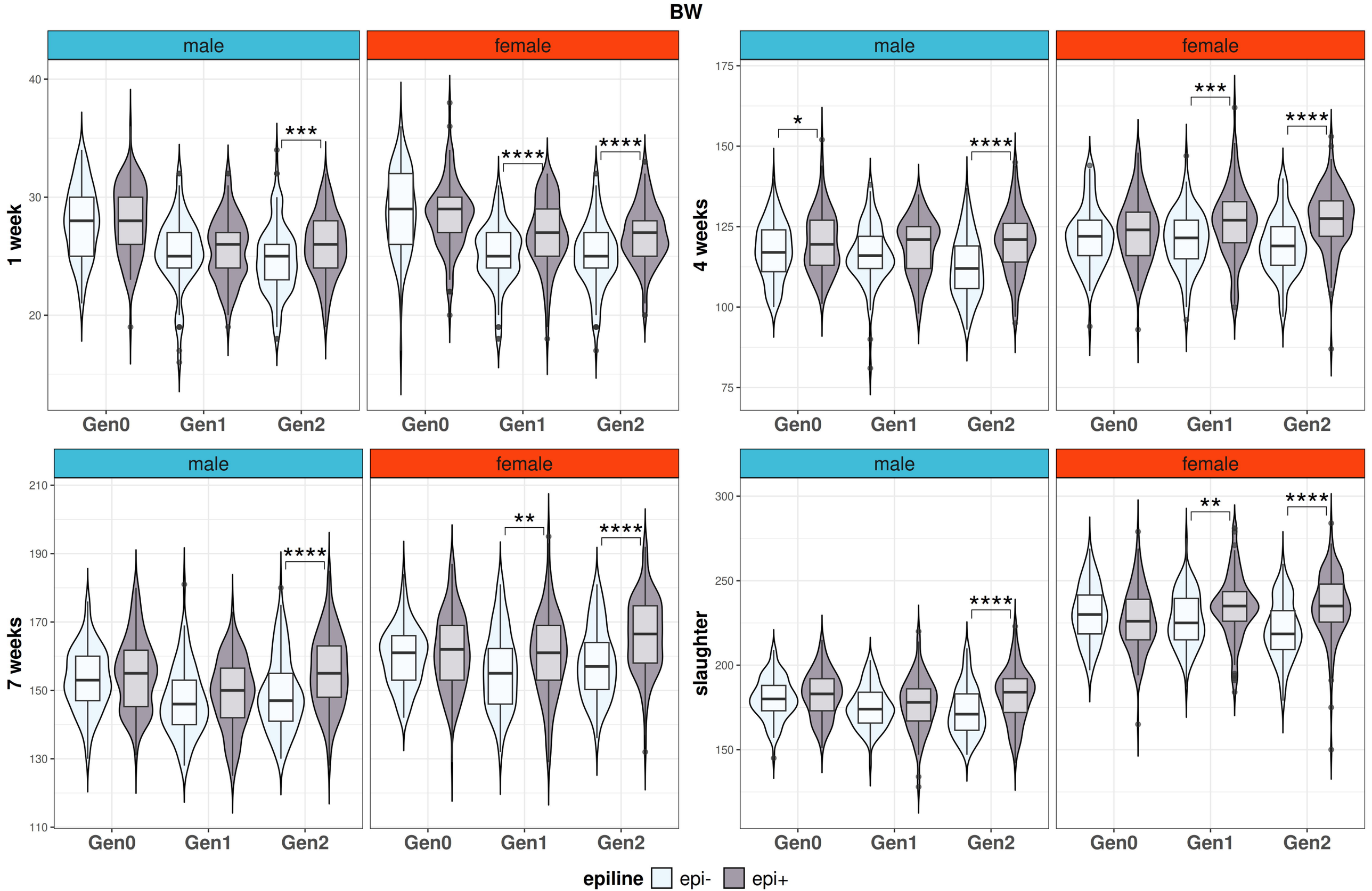

BHV
male
female
total distance
velocity
freq. at centre
freq. at periphery
duration at centre
duration at periphery
Gen0
Gen1
Gen2
*
epiline
epi-
epi+
pwc: Tukey HSD; p.adjust: Tukey

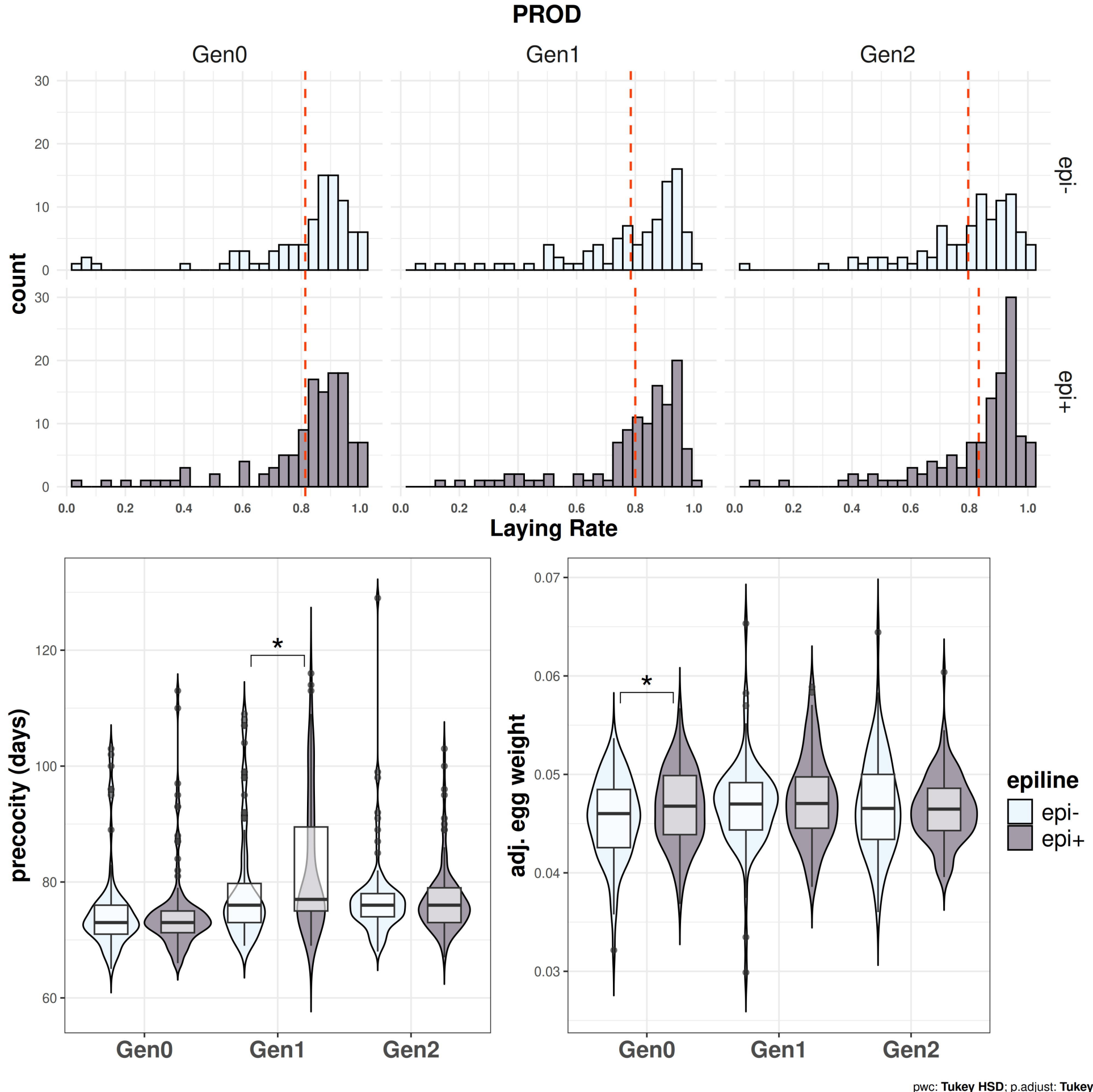

Gen0
Gen1
Gen2
female
male
mean BW (g)
Age (weeks)
epiline
epi-
epi+
sex
male
female

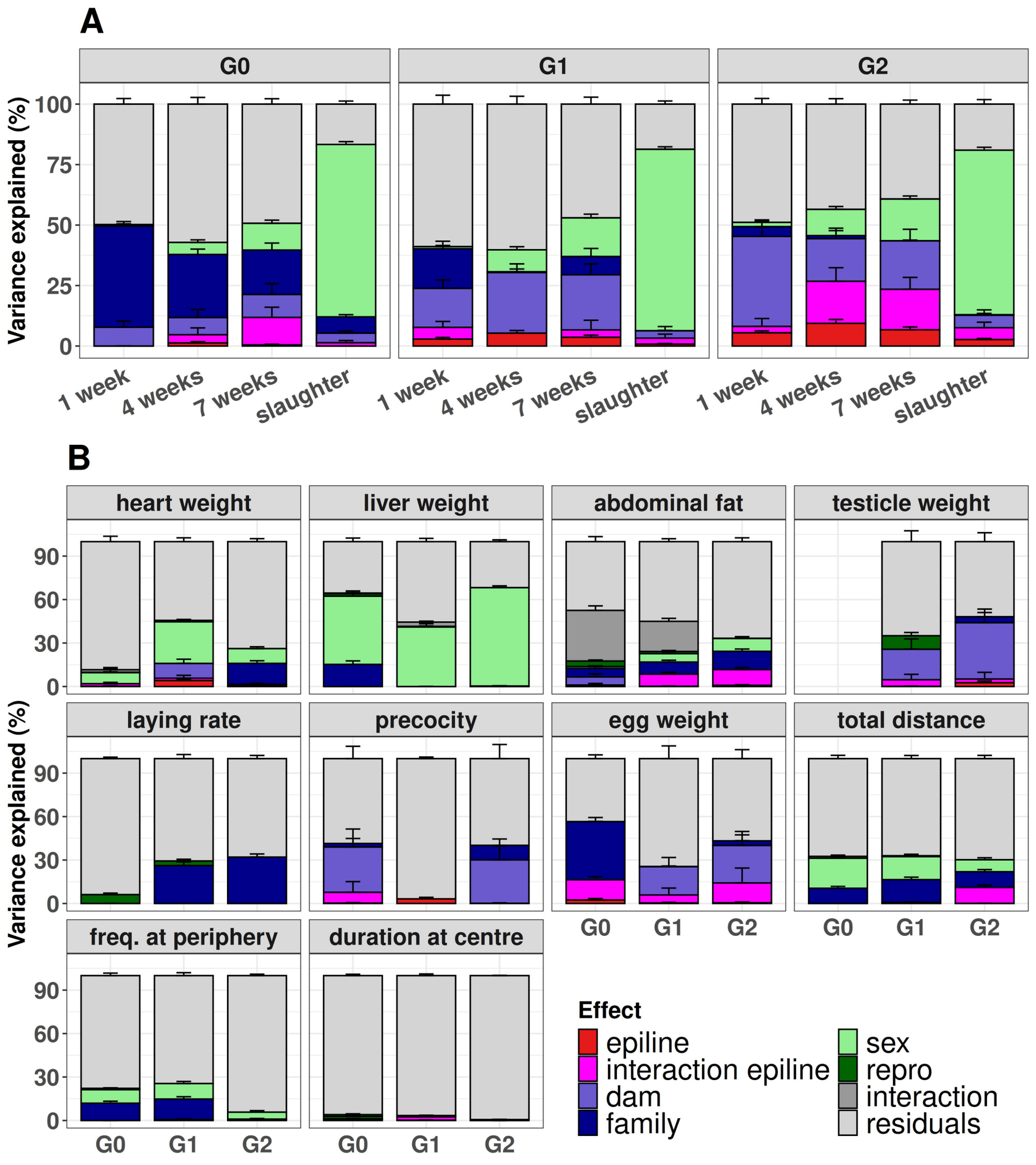


pwc: **Tukey HSD**; p.adjust: **Tukey**


20 founding families
genistein
genistein
genistein
genistein
...
G-1
G0
G1
G2
Legend:
founding family combination
epi -
epi +


BW
male
female
1 week
4 weeks
7 weeks
slaughter
Gen0
Gen1
Gen2
epiline
epi-
epi+
pwc: Tukey HSD; p.adjust: Tukey


PROD
Gen0
Gen1
Gen2
epi-
epi+
count
Laying Rate
precocity (days)
adj. egg weight
epiline
epi-
epi+
*
*
pwc: Tukey HSD; p.adjust: Tukey


A
G0
G1
G2
Variance explained (%)
100
75
50
25
0
1 week
4 weeks
7 weeks
slaughter
B
heart weight
liver weight
abdominal fat
testicle weight
laying rate
precocity
egg weight
total distance
freq. at periphery
duration at centre
90
60
30
0
Effect
epiline
interaction epiline
dam
family
sex
repro
interaction
residuals