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RESEARCH ARTICLE

Effects of crossbreeding among geographically distinct *Tenebrio molitor* populations on phenotypic performance and developmental traits

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Abstract

The yellow mealworm (*Tenebrio molitor*) is a key species for sustainable insect production; however, the phenotypic consequences of crossbreeding among geographically distinct populations remain insufficiently characterised. In this study, we evaluated 16 crosses involving populations from Spain, China, South Korea and England under standardised laboratory conditions. Adult phenotypic traits assessed included body weight, body length, cuticle coloration (RGB-based index) and malformation incidence. Significant phenotypic differences were detected among cross types, particularly for adult body weight, which showed the highest discriminatory power among traits. Crosses between populations tended to be associated with higher body weight values and lower malformation frequencies compared to within-population crosses. In addition, cuticle coloration showed a moderate association with malformation incidence ($\rho = 0.557$), suggesting a potential relationship between coloration patterns and developmental variation. Multivariate analyses indicated that body size traits explained most of the observed phenotypic variation among crosses, whereas coloration contributed more weakly and appeared partially independent from the main axes of variation. The results reveal consistent phenotypic patterns associated with cross origin under controlled rearing conditions. However, the present study does not allow direct inference of underlying genetic mechanisms and environmental or developmental factors may also contribute to the observed variation. These findings provide preliminary insights that may support future breeding strategies and phenotypic screening approaches for *T. molitor* production systems.

Keywords

body weight – colouration – crossbreeding – insect production – phenotypic variation – *Tenebrio molitor*

1 Introduction

Phenotypic variation is an important determinant of performance and adaptability in insects, particularly under artificial rearing and domestication. The yellow mealworm, *Tenebrio molitor*, has emerged as a model species for sustainable food and feed production due to its high nutritional value, efficient feed conversion and capacity to utilise low-value organic substrates (Oonincx and de Boer, 2012; Rumpold and Schlüter,

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2013; van Huis, 2013). As the insect production sector expands, improving consistency, productivity and robustness of reared populations has become a major objective (Mancini *et al.*, 2019; van Huis, 2020).

To date, most research in *T. molitor* has focused on environmental and nutritional factors influencing growth, survival and microbiota composition (Adámková *et al.*, 2020; Dillon and Dillon, 2004; Garofalo *et al.*, 2019; Ribeiro *et al.*, 2018; Vigneron *et al.*, 2019). In contrast, the role of population origin and crossbreeding in shaping phenotypic variation remains comparatively underexplored. In other insect systems, crosses between differentiated populations have been shown to influence growth-related traits, developmental outcomes and phenotypic variability, although the magnitude and direction of these effects depend strongly on the biological context and experimental design (Arisa-Marín and de Luna, 2020; Edmands, 2007).

Commercial and laboratory populations of *T. molitor* differ in their origin, management history and potential exposure to selection pressures. These differences may contribute to phenotypic variability among populations, but their consequences under controlled crossbreeding conditions remain insufficiently characterised. A systematic evaluation of phenotypic traits across crosses is therefore necessary to identify patterns of variation that may be relevant for breeding and production contexts.

Traits such as body weight, body length and developmental anomalies are directly linked to production efficiency and quality in insect farming systems. In addition, cuticle coloration, although less studied, may provide complementary information on phenotypic variation and has been proposed as a potential indicator of physiological or developmental status in insects. However, its relevance and consistency under controlled rearing conditions remain unclear.

In this study, we examine phenotypic variation among crosses derived from four geographically distinct *Tenebrio molitor* populations from Spain, England, China and South Korea. We assess body weight, body length, cuticle coloration and malformation incidence in both homogeneous and heterogeneous crosses. Homogeneous crosses refer to within-population matings (e.g. S × S), whereas heterogeneous crosses refer to between-population matings (e.g. S × C). These terms are used descriptively and do not imply specific levels of genetic diversity or inbreeding.

We test whether cross origin is associated with differences in phenotypic traits under standardised conditions. Specifically, we evaluate whether crosses between geographically distinct populations tend to show contrasting phenotypic patterns compared to within-population crosses. This multi-trait assessment aims to provide a phenotypic framework to support early-stage screening and the development of breeding strategies for large-scale mealworm production.

2 Materials and methods

Experimental design and crosses

The aim of this study was to evaluate phenotypic variation among crosses derived from geographically distinct populations of *Tenebrio molitor* under standardised rearing conditions.

Adult *T. molitor* specimens were collected from four geographically distinct origins: (i) Spain: C. Esparragal 18, 47155 Santovenia de Pisuerga, Valladolid; (ii) England: 60–61 Barnes High St, London SW13 9LF; (iii) P.R. China: Xiacheng District, Hangzhou, Zhejiang (310004); and (iv) South Korea: 6-1 Nangmin-ro, Nangmin-dong, Dongnae-gu, Busan. Prior to the experiment, all populations were maintained separately for at least three generations under identical laboratory conditions to minimise environmental and maternal carry-over effects. These populations are therefore referred to as parental populations (F0).

A full crossing scheme was implemented, including four homogeneous crosses (S × S, C × C, K × K, E × E) and twelve heterogeneous crosses, including reciprocals (S × C, C × S, S × K, K × S, S × E, E × S, C × K, K × C, C × E, E × C, K × E, E × K) (Table 1). This resulted in a total of 16 experimental groups, which were treated as independent phenotypic units for analysis.

Each cross was established using three independent replicate units, each consisting of 10 mating pairs (1 male: 1 female) randomly selected from the corresponding parental populations. Adults were sexed based on morphological characteristics of the abdominal sternites and genital morphology under a stereomicroscope. Only newly emerged adults (2–5 days post-emergence) were used to standardise reproductive status and minimise age-related variability. Oviposition was allowed for 7 days, after which adults were removed. Eggs from each replicate were collected and reared separately to maintain independence among replicates. For larval development, individuals derived from each replicate were reared under standardised density conditions (approximately 300 larvae per container), ensuring comparable rearing environments across all crosses. Survival during the experimental period was high across all treatments and no substantial differences in mor-

TABLE 1 Experimental crossing scheme among geographically distinct *Tenebrio molitor* populations

Male origin	Female origin	Cross
Spain	Spain	S × S
Spain	China	S × C
China	Spain	C × S
Spain	South Korea	S × K
South Korea	Spain	K × S
Spain	England	S × E
England	Spain	E × S
China	China	C × C
China	South Korea	C × K
South Korea	China	K × C
China	England	C × E
England	China	E × C
South Korea	South Korea	K × K
South Korea	England	K × E
England	South Korea	E × K
England	England	E × E

The experimental design included four homogeneous crosses (within-population matings: S × S, C × C, K × K and E × E) and twelve heterogeneous crosses (between-population matings), including reciprocal combinations (e.g. S × C and C × S). Each cross was established using independent replicate units under standardised laboratory conditions. Reciprocal crosses were included to account for potential parental effects but were pooled for statistical analyses. Population codes: S, Spain; C, P.R. China; K, South Korea; E, England.

tality were observed among crosses. Reciprocal crosses were included to account for potential parental effects; however, they were pooled for statistical analysis (see below).

Rearing conditions

All experimental units were maintained in a climate-controlled chamber under standardised conditions (Table 2). Temperature was maintained at 25 ± 1 °C, relative humidity at 60 ± 5% and photoperiod at 4 h light: 20 h dark. To minimise positional effects within the chamber, containers were randomly distributed and periodically rotated throughout the experiment.

Larvae were reared in ventilated plastic containers (500 ml) containing wheat bran as substrate and provided with fresh carrot slices as a moisture source (renewed every 3 days). Larval density was standardised to approximately 300 individuals per container to ensure comparable rearing conditions across crosses.

Larvae were maintained under these conditions until adulthood, when phenotypic measurements were performed. Although variation in oviposition timing may have introduced some degree of age heterogeneity, all crosses were handled identically and this source of variation is assumed to have been randomly distributed among treatments.

All experimental units were maintained within the same controlled chamber and containers were randomly distributed and periodically rotated to minimise positional and microclimatic effects.

Phenotypic measurements

Phenotypic traits were quantified at the adult stage under standardised rearing conditions. For measurements, 30 adult individuals per cross (10 per replicate) were randomly selected at an early post-emergence stage to ensure comparability. Prior to measurement, adults were euthanised and oven-dried to minimise variation associated with body water content. Adult weight (g) was measured using a precision balance (±0.001 g) and body length (cm) was recorded from the anterior margin of the head to the posterior tip of the abdomen using digital callipers (Figure 1).

Malformation incidence was assessed visually in adults and recorded as a binary variable (0 = absent, 1 = present), with individuals classified into predefined categories including abdominal constrictions, asymmetrical body curvature and underdeveloped appendages. All observations were conducted under consistent magnification and lighting conditions and due to the relatively low frequency of malformations in some groups, this variable was interpreted cautiously as an exploratory indicator.

Cuticle coloration was quantified using a standardised digital imaging protocol with controlled illumination, background and acquisition settings. A triangular region defined by three anatomical landmarks on the elytra was used as a reference area (Figure 2), from which RGB values were extracted at the centroid using a fixed circular sampling region (25-pixel radius). The mean RGB value (average of red, green and blue channels) was used as an integrative index of coloration. Given that RGB is not a perceptually uniform colour space and may be sensitive to subtle variations in illumination and cuticle state, this metric should be interpreted as a relative proxy of coloration under controlled conditions rather than a comprehensive descriptor of colour.

All measurements were performed by the same operator following a consistent protocol to minimise procedural variability, although biological factors such as

TABLE 2 Standardised rearing conditions applied to all experimental crosses of *Tenebrio molitor*

Parameter	Condition
Temperature	25 ± 1 °C
Relative humidity	60 ± 5%
Photoperiod	4 h light: 20 h dark
Substrate	Wheat bran
Moisture source	Fresh carrot (renewed every 3 days)
Larval density	approx. 300 larvae per container
Container type	500 ml ventilated plastic containers
Chamber management	Randomised container distribution and periodic rotation

All crosses were maintained under controlled environmental conditions within the same climate chamber. Temperature and relative humidity are presented as target values with controlled variation. Containers were randomly distributed and periodically rotated to minimise positional and microclimatic effects, ensuring comparable rearing conditions across all experimental groups.

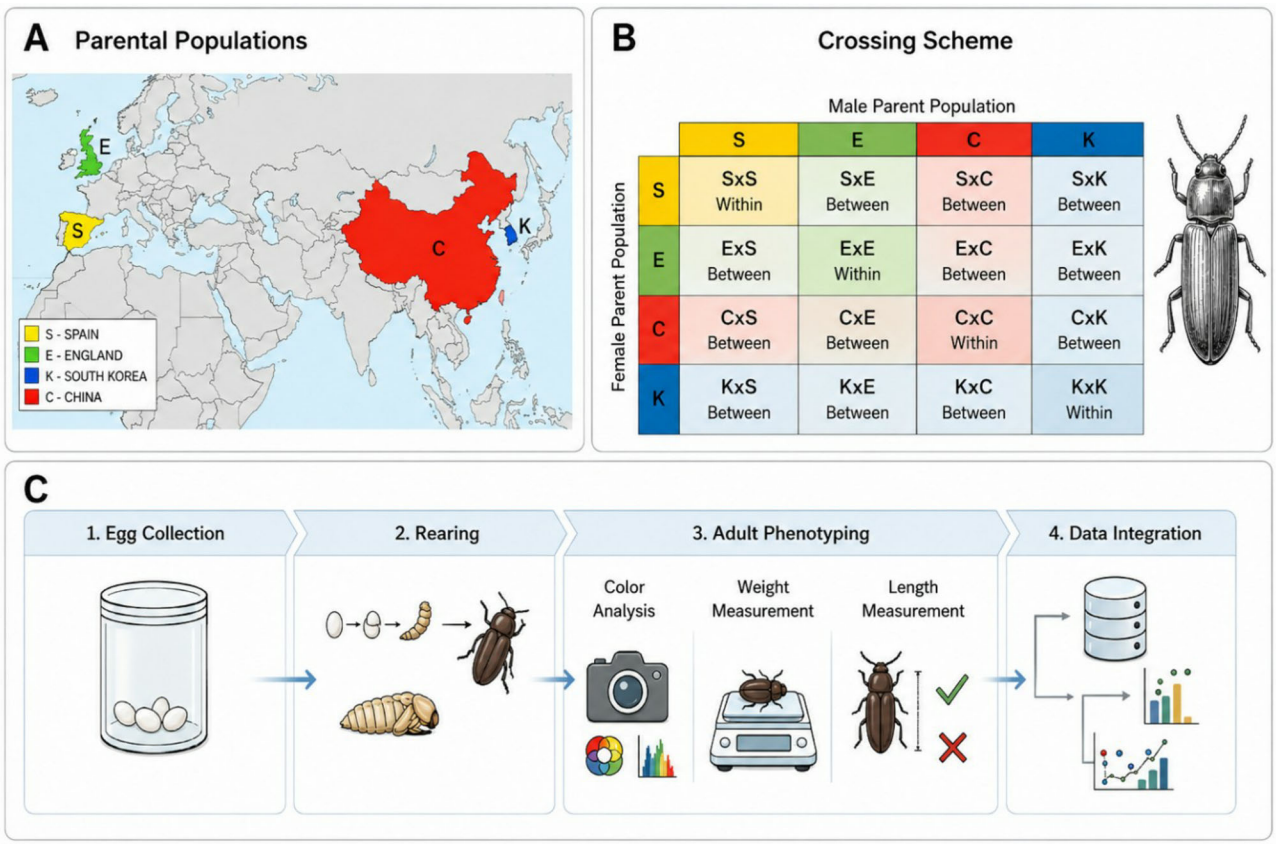


FIGURE 1 Experimental design and crossing strategy among four geographically distinct *Tenebrio molitor* populations. (A) Geographic origin of the four parental populations used in this study: Spain (S), England (E), P.R. China (C) and South Korea (K). (B) Crossing scheme including homogeneous crosses (within-population; diagonal cells) and heterogeneous crosses (between populations; off-diagonal cells). Reciprocal crosses (e.g. S × C and C × S) were included in the experimental design but pooled for statistical analyses. (C) Overview of the experimental workflow, including egg collection, establishment of reciprocal crosses, rearing under standardised laboratory conditions, adult phenotyping and data integration. Phenotypic traits measured in adults included body weight, body length, cuticle coloration (RGB index) and malformation incidence. The figure is intended to illustrate the experimental design and measured variables and does not represent sample size or statistical relationships.

degree of cuticle sclerotisation may introduce residual variation, particularly in colour-related traits.

Statistical analysis

All statistical analyses were performed in R v4.3.1 (R Core Team, 2023). Prior to analysis, data distri-

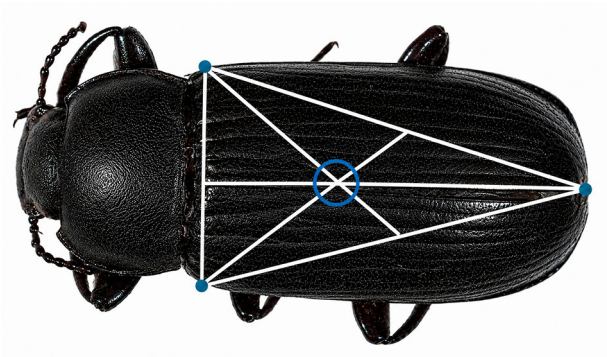


FIGURE 2 Standardised anatomical landmarks and reference area used for cuticle coloration analysis in adult *Tenebrio molitor* (dorsal view). Blue points indicate the three anatomical landmarks used to define the reference region on the elytra: anterior dorsal insertion point, posterior dorsal insertion point and elytral apex. White lines delimit the triangular reference area used for geometric standardisation. The central blue circle represents the centroid of the polygon, from which RGB values were extracted using a fixed circular sampling region. This standardised imaging framework was used to quantify cuticle coloration under controlled illumination and acquisition conditions.

butions were evaluated using Shapiro–Wilk tests for normality and Levene's tests for homogeneity of variance. As several variables deviated from normality and heteroscedasticity was detected in some cases, nonparametric approaches were primarily employed.

Differences among crosses for quantitative traits (adult body weight, adult body length and colour index) were assessed using the Kruskal–Wallis test. When significant effects were detected, pairwise comparisons were conducted using Dunn's post hoc test with Bonferroni correction to control for familywise error rates. This conservative approach was selected to minimise type I error associated with multiple group comparisons.

Associations among traits were evaluated using Spearman's rank correlation coefficient (ρ), which is appropriate for non-normally distributed data. In particular, the relationship between colour index and malformation incidence was explored as an exploratory analysis.

Body condition was evaluated using analysis of covariance (ANCOVA), with body weight as the dependent variable, body length as a covariate and cross as a fixed factor. The homogeneity of slopes assumption was verified prior to interpretation and adjusted means were compared only when model assumptions were met.

Malformation incidence, treated as a binary response variable, was analysed using generalised linear models (GLMs) with a binomial error distribution. Overall differences among crosses were evaluated using likelihood ratio tests and odds ratios with 95% confidence intervals were calculated to estimate effect sizes. Given the relatively low frequency of malformations in several groups, these results were interpreted cautiously.

To summarise multivariate phenotypic variation, a principal component analysis (PCA) was performed on centred and scaled variables. Components with eigenvalues greater than 1 were retained and Varimax rotation was applied to improve interpretability of component loadings. Factor loadings were examined to identify the traits contributing most strongly to each axis. The PCA was used as a descriptive tool to visualise clustering patterns among crosses rather than to infer underlying biological mechanisms.

Reciprocal crosses (e.g. S × C and C × S) were pooled for statistical analyses because preliminary exploration did not reveal consistent directional differences. However, potential maternal or parental effects cannot be excluded and therefore represent a limitation of the study.

All tests were evaluated at a significance threshold of $\alpha = 0.05$. Effect sizes and confidence intervals were reported where appropriate to complement p-values. No data points were excluded from statistical analyses; extreme values were removed only for graphical visualisation purposes. Given the experimental design, the results should be interpreted as reflecting phenotypic patterns under controlled conditions and caution is required when attributing these patterns to specific genetic mechanisms.

Ethical considerations

All procedures complied with institutional and international guidelines for the ethical use of insects in research.

3 Results

Summary statistics were obtained for the three quantitative variables (colour, weight and length) and for malformation incidence. Violin plots were used to visualise distributional patterns for each variable across all crosses (Figures 3–5).

Colouration

Adult cuticle coloration differed significantly among crosses (Kruskal–Wallis $H = 101.3$, df = 15, $p < 0.001$). Differences in the colour index (mean RGB value) were observed across cross types, with homogeneous crosses, particularly S × S and C × C, generally show-

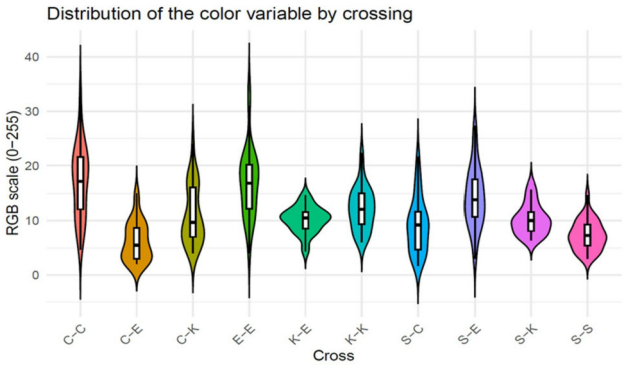


FIGURE 3 Distribution of cuticle coloration across *Tenebrio molitor* cross types. Violin plots represent the distribution of the colour index (mean RGB value) based on individual measurements. The width of each violin reflects the kernel density of the data and horizontal lines indicate median values. Outliers were removed for visualisation purposes only and were retained in all statistical analyses. A total of $N = 1440$ individuals were included.

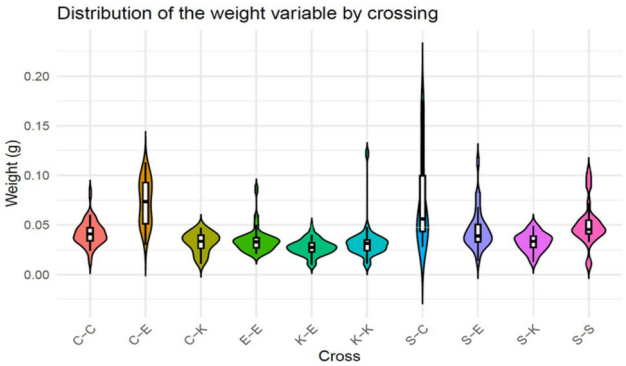


FIGURE 4 Distribution of body weight across *Tenebrio molitor* cross types. Violin plots represent the distribution of individual body weight values for each cross. The width of each violin reflects the kernel density of the data and horizontal lines indicate median values. Outliers were removed for visualisation purposes only and were retained in all statistical analyses.

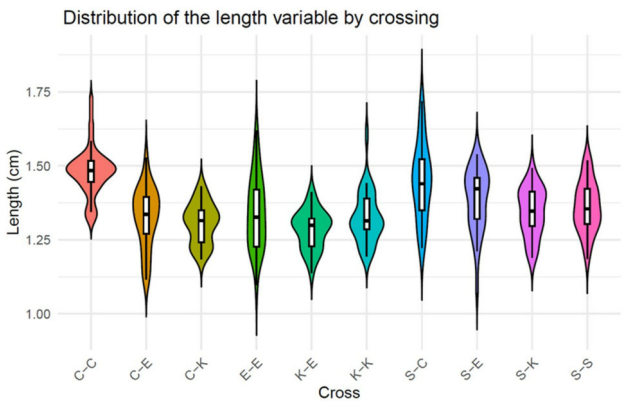


FIGURE 5 Distribution of body length across *Tenebrio molitor* cross types. Violin plots represent the distribution of individual body length values for each cross. The width of each violin reflects the kernel density of the data, and horizontal lines indicate median values. Outliers were removed for visualisation purposes only and were retained in all statistical analyses.

ing more extreme coloration values, whereas heterogeneous crosses tended to exhibit intermediate values. The highest mean coloration values and greatest variability were observed in the C × C cross, while lower values were recorded in combinations such as C × K. In contrast, several heterogeneous crosses (e.g. S × C and K × E) displayed narrower interquartile ranges and reduced dispersion in the violin plots, indicating more homogeneous distributions of the colour index (Figure 3). Homogeneous crosses showed broader distributions and more extreme values, whereas heterogeneous crosses tended to present more moderate and less variable coloration patterns.

A positive association was detected between the colour index and malformation incidence (Spearman's $\rho = 0.55$, $p < 0.001$). However, given the limitations of the RGB-based metric and the observational nature of this analysis, this relationship should be interpreted cautiously as an exploratory pattern rather than as evidence of a direct physiological link.

Growth and size traits

Significant variation in adult body weight and body length was observed among the 16 cross types. Crosses involving Chinese parental lines, particularly C × E and S × C, were generally associated with higher values for both traits, as reflected by higher central tendencies and broader distributions (Figure 4). The highest mean adult body weight was recorded in the C × E cross (0.122 ± 0.004 g), whereas the lowest values were observed in K × K and S × S crosses. Adult body weight and body length were moderately correlated (Spearman's $\rho = 0.447$, $p < 0.001$), indicating a consistent scaling relationship between these traits across crosses.

Distributional patterns also differed among crosses. The C × E cross exhibited a tendency towards positive skewness, whereas the homogeneous C × C cross showed a tendency towards negative skewness, reflecting a concentration of individuals at higher values (Figure 5). Differences in dispersion were also evident: K × K and C × C displayed relatively narrow distributions, while S × S and S × C showed greater variability in adult body weight (Figure 4).

Adult body length followed a similar overall pattern, with the highest values observed in C × E and the lowest in C × C. Crosses involving Korean parental lines, particularly K × K and K × E, tended to display more compact distributions, whereas S × C showed greater variability.

TABLE 3 Summary statistics for adult phenotypic traits across *Tenebrio molitor* cross types

Trait	Highest mean values	Lowest mean values	Significant differences	Notable patterns/remarks
Adult weight (g)	C × E, S × C	K × K	$p < 0.05$ (68.9% of comparisons)	Crosses involving Chinese ancestry generally showed higher body weight values.
Adult length (cm)	C × E	C × C	$p < 0.05$ (51.1%)	Crosses involving Chinese ancestry tended to produce larger adults.
Colouration (RGB index)	C × C	C × K	$p < 0.05$ (60%)	Homogeneous crosses showed broader and more extreme colour distributions.
Malformation incidence (%)	C × C, S × S	S × K, S × E, C × K, K × E	Logistic regression, $p < 0.001$	Patterns suggested increased occurrence of malformations in some homogeneous crosses

Summary of descriptive patterns for adult body weight, adult body length, cuticle coloration (RGB index) and malformation incidence across cross types. Values correspond to adult measurements obtained under standardised laboratory conditions. Descriptive trends are summarised based on the statistical analyses presented in the Results section.

These results reveal consistent differences in the distribution of growth-related traits among crosses under the experimental conditions.

Presence of malformations

The incidence of visible developmental malformations differed significantly among cross types (logistic regression, $\chi^2 = 84.9$, df = 15, $p < 0.001$). Higher frequencies were observed in homogeneous crosses, particularly S × S and C × C, whereas several heterogeneous combinations (e.g. S × K, S × E, C × K, K × E and C × E) showed low or no recorded malformations. Odds ratio estimates indicated that individuals from homogeneous crosses were more likely to exhibit malformations than those from heterogeneous crosses (odds ratio = 3.4, 95% CI: 2.2–5.1, $p < 0.001$). However, given the relatively low frequency of malformations in several groups and the observational nature of this assessment, these results should be interpreted with caution. These findings reveal consistent differences in malformation incidence among cross types under the experimental conditions, but they do not allow direct inference of underlying mechanisms or developmental processes.

Summary of descriptive patterns

A synthesis of the main trends is presented in Table 3. Crosses involving Chinese ancestry tended to be associated with higher body weight and length values compared to other combinations. In contrast, heterogeneous crosses generally showed narrower distributions of colour values and lower frequencies of malformations, whereas homogeneous crosses, particularly C × C and S × S, were associated with higher malformation rates and more extreme coloration values. These observations represent consistent descriptive patterns across the dataset and are supported by the inferential and multivariate analyses presented above, although they should be interpreted as phenotypic trends under controlled conditions rather than as direct evidence of underlying genetic mechanisms.

Normality tests

Normality assessments using the Shapiro–Wilk test indicated that several variables deviated from a normal distribution. Consequently, non-parametric methods were applied for comparisons among crosses. Significant differences were detected among cross types for all quantitative traits (colour index, body weight and body length) using the Kruskal–Wallis test ($p < 0.05$).

Post hoc pairwise comparisons using Dunn's test with Bonferroni correction identified multiple significant differences among crosses. The proportion of significant pairwise comparisons varied among traits, with body weight showing the highest percentage (68.9%), followed by colour index (60%) and body length (51.1%) (Table 4).

Patterns of pairwise differentiation were not uniformly distributed across crosses. Combinations involving Chinese ancestry (e.g. C × E and S × C) were more frequently involved in significant contrasts for at least one trait, whereas several crosses involving Korean individuals showed fewer significant differences. These results

TABLE 4 Proportion of significant pairwise differences among *Tenebrio molitor* cross types for each adult phenotypic trait

Trait	Significant pairwise comparisons	Percentage of significant comparisons
Adult body weight	62/90	68.9%
Cuticle coloration (RGB index)	54/90	60.0%
Adult body length	46/90	51.1%

Values represent the number and percentage of pairwise comparisons showing significant differences among crosses based on Dunn's post hoc test with Bonferroni correction following Kruskal–Wallis analyses ($p < 0.05$). Comparisons were performed for adult body weight, adult body length and cuticle colouration (RGB-based colour index).

TABLE 5 Spearman's rank correlation coefficients (ρ) and associated p-values among adult phenotypic traits in *Tenebrio molitor*

Trait pair	ρ	*p*-value
C–W	−0.213	<0.05
C–L	0.091	n.s.
W–L	0.447	<0.001
C–M	0.557	<0.001
W–M	0.479	<0.001
L–M	0.453	<0.001

Pairwise correlations were calculated between cuticle coloration (C; RGB-based colour index), body length (L), body weight (W) and malformation incidence (M). Correlation coefficients (ρ) indicate the strength and direction of the associations and corresponding p-values are provided to assess statistical significance.

reflect variability in the degree of phenotypic differentiation among cross types under the experimental conditions.

Correlation between variables

Spearman's rank correlations among phenotypic traits are presented in Table 5. The strongest association was observed between the colour index and malformation incidence ($\rho = 0.557$), indicating that higher values of the colour index were associated with an increased frequency of malformations. Body weight and body length showed a moderate positive correlation ($\rho = 0.447$), consistent with their expected allometric relationship. In contrast, the association between colour index and body weight was weak and negative. These correlations describe patterns of association among traits under the experimental conditions and should be interpreted as descriptive relationships rather than evidence of direct functional or causal links.

Body condition (ANCOVA)

An analysis of covariance (ANCOVA) was performed to evaluate body condition, with body weight as the dependent variable, body length as a covariate and cross as a fixed factor. Significant effects were detected for body length ($p < 0.001$), cross ($p < 0.01$) and their interaction ($p < 0.01$) (Table 6), indicating that the relationship between weight and length varied among crosses. Adjusted means revealed differences among cross types after accounting for body size. Higher adjusted weight values were observed in crosses such as C × E, followed by S × C and S × S, whereas lower values were recorded in combinations involving Korean parental lines (e.g. K × K and K × E). These differences are illustrated in Figure 6.

TABLE 6 Results of ANCOVA examining adult body condition across *Tenebrio molitor* cross types

	F	df	*p*
Length	67.22	1.19	<0.001
Cross	2.90	9.19	<0.01
Length × Cross	2.95	9.19	<0.01

Body weight was used as the dependent variable, body length as a covariate and cross as a fixed factor. The analysis tests for differences in weight among crosses after adjusting for body size.

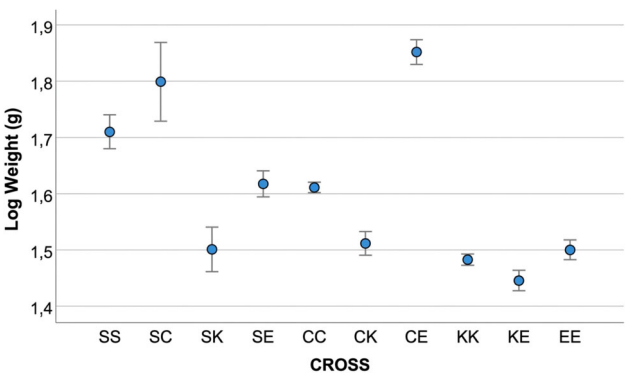


FIGURE 6 Comparison of adult body condition among *Tenebrio molitor* cross types. Points represent size-adjusted mean body weight (±95% confidence intervals) estimated from ANCOVA, with body length included as a covariate. Differences among crosses reflect variation in body weight after accounting for body size.

TABLE 7 Rotated component matrix for the principal component analysis (PCA) of adult phenotypic traits in *Tenebrio molitor*

Variable	PC1	PC2
Adult body weight	0.912	0.184
Adult body length	0.887	0.221
Cuticle coloration (RGB index)	0.006	0.931
Malformation incidence	0.318	0.742
Explained variance (%)	49.74	36.28
Cumulative variance (%)	49.74	86.01

Loadings are shown for the two principal components with eigenvalues greater than 1. PC1 was primarily associated with body size traits, whereas PC2 was mainly related to cuticle coloration and malformation incidence. Values represent rotated factor loadings obtained after Varimax rotation.

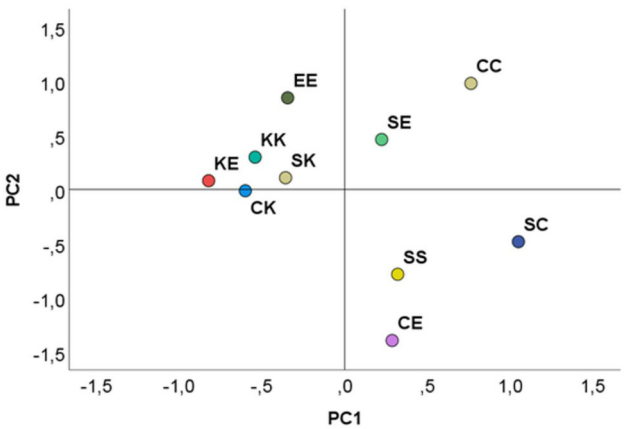


FIGURE 7 Principal component analysis (PCA) ordination of *Tenebrio molitor* crosses based on phenotypic traits. Points represent the centroids of each cross calculated from individual factor scores on the first two principal components (PC1 and PC2). The ordination summarises multivariate phenotypic variation among crosses, with axes corresponding to the main components identified in the PCA.

Principal component analysis (PCA)

The principal component analysis (PCA) explained 86.01% of the total variance using the first two components (PC1: 49.74%; PC2: 36.28%). PC1 was primarily associated with body weight and body length, whereas PC2 captured most of the variation related to the colour index (Table 7). The PCA ordination revealed patterns of clustering among crosses (Figure 7). Crosses involving Korean parental lines tended to occupy lower PC1 values, reflecting smaller body size and weight, while crosses involving Chinese ancestry were generally positioned towards higher PC1 values. Some crosses, such as S × C, showed relatively high scores on both axes, indicating combined variation in size-related traits and coloration. The loading of the colour index on PC1 was close to zero (0.006), indicating that coloration contributed minimally to the primary axis of variation, which was dominated by size-related traits. This suggests that colour variation is largely independent of the main patterns of phenotypic differentiation captured by PC1. The PCA provides a descriptive summary of multivariate phenotypic variation among crosses under the experimental conditions and should not be interpreted as evidence of underlying biological or genetic mechanisms.

4 Discussion

The present study reveals consistent phenotypic differences among *Tenebrio molitor* crosses under standardised rearing conditions, particularly for growth-related traits and coloration. However, these patterns should be interpreted as phenotypic responses associated with cross origin rather than as direct evidence of underlying genetic mechanisms.

Differences in adult weight and length among crosses indicate substantial variation in growth-related traits. Crosses involving Chinese parental lines were generally associated with higher body size values, whereas crosses involving Korean parental lines tended to show lower values. These differences may reflect variation in the origin and history of the populations used, including potential differences in domestication, maintenance conditions, or prior selection, although such factors were not directly assessed in this study (Adamaki-Sotiraki *et al.*, 2023b; Mancini *et al.*, 2019). Previous studies have reported moderate to high phenotypic variability and heritability for growth-related traits in *T. molitor*, supporting the relevance of such variation for breeding contexts (Sellem *et al.*, 2024; Morales-Ramos *et al.*, 2025).

The moderate positive correlation between body weight and length is consistent with expected allometric relationships in insects (Mocq *et al.*, 2024; Araújo Foerster *et al.*, 2024) and reflects coordinated growth processes. Differences in size-adjusted weight (body condition) among crosses further indicate that phenotypic variation is not limited to size alone but also involves proportional relationships between traits. Nevertheless, given the experimental design, these patterns should be interpreted cautiously, as environmental and developmental factors may also contribute to the observed variation.

Multivariate analyses (PCA) provided a descriptive summary of phenotypic variation among crosses, with body size traits contributing most strongly to the primary axis of variation. Although some grouping patterns were observed, these should be considered exploratory and do not imply discrete genetic structuring. The limited contribution of the colour index to the main axes of variation further supports the view that coloration represents a partially independent phenotypic dimension.

Differences in malformation incidence among crosses followed a consistent pattern, with higher frequencies observed in homogeneous crosses and lower frequencies in several heterogeneous combinations. Although statistical support was limited in some comparisons, this pattern is broadly consistent with previous studies describing the effects of reduced genetic diversity on developmental and physiological traits in animal populations (Charlesworth and Willis, 2009; Frankham, 2005). However, the relatively low occurrence of malformations in several groups and the observational nature of the analysis limit the strength of inference and these results should be interpreted cautiously. Comparable effects of reduced genetic diversity on behavioural and physiological traits have also been reported in *T. molitor* (Pölkki *et al.*, 2012), although the extent to which such mechanisms underlie the present observations cannot be determined.

Variation in cuticle coloration among crosses was also evident, with homogeneous crosses tending to show more extreme values and heterogeneous crosses more intermediate distributions. The observed association between coloration and malformation incidence suggests a potential relationship between these traits; however, given the limitations of the RGB-based metric and the exploratory nature of this analysis, this relationship should not be interpreted as evidence of a direct physiological or developmental link.

Studies in other insect systems, including *Bombyx mori* and *Apis mellifera*, have shown that crosses between differentiated populations can influence growth performance, developmental traits and phenotypic variability (Edmands, 2007; Arisa-Marín and de Luna, 2020). In this context, the patterns observed here are broadly consistent with previous findings, although direct comparisons are limited by differences in experimental design and biological systems.

From an applied perspective, the results highlight that phenotypic variation among crosses can be detected under controlled conditions and may be relevant for early-stage screening in breeding programs. Traits such as body weight and coloration could provide practical indicators for comparing performance among lines. However, the present study does not allow evaluation of long-term stability, heritability, or performance under production conditions, which are essential for assessing the utility of these traits in industrial breeding (Gowda *et al.*, 2025; Sandrock *et al.*, 2025).

The findings indicate that cross origin is associated with measurable differences in phenotypic traits in *T. molitor*. Further work incorporating larger population sizes, controlled breeding designs and multigenerational analyses will be required to disentangle genetic and environmental contributions and to evaluate the practical implications of these patterns for insect production systems (van Huis, 2020).

5 Conclusions

The present study shows that crosses among geographically distinct *Tenebrio molitor* populations are associated with consistent differences in adult phenotypic traits under standardised laboratory conditions. Variation in body weight, body length, cuticle coloration and malformation incidence was detected among cross types, indicating that cross origin may contribute to observable phenotypic diversity in this species.

Crosses between populations generally tended to show higher body size values and lower malformation frequencies than within-population crosses, although these patterns should be interpreted cautiously and cannot be directly attributed to specific genetic mechanisms. Likewise, the association observed between cuticle coloration and malformation incidence suggests a potential relationship between traits, but its biological significance remains unclear and requires further investigation.

From an applied perspective, the results indicate that phenotypic screening across crosses may provide useful preliminary information for the evaluation of *T. molitor* lines in breeding programs. In particular, adult body weight and coloration-related traits may represent practical indicators for comparative assessments under controlled rearing conditions.

However, the present study was limited to a single generation and standardised experimental conditions. Future studies incorporating larger population sizes, multigenerational approaches and additional performance-related traits will be necessary to evaluate the stability, heritability and practical relevance of the

observed phenotypic patterns for industrial insect production systems.

6 Future prospects

Future research should focus on evaluating the consistency and reproducibility of the phenotypic patterns observed among *Tenebrio molitor* crosses under different rearing conditions and across multiple generations. In particular, studies incorporating larger sample sizes, replicated breeding designs and long-term monitoring will be necessary to assess the stability of differences among cross types and to better distinguish potential genetic and environmental contributions to phenotypic variation.

Controlled crossing schemes, including reciprocal crosses and backcrosses, may help clarify the influence of parental effects and inheritance patterns on growth-related and developmental traits. In parallel, the integration of molecular approaches such as genomic and transcriptomic analyses could provide additional insight into the biological basis of variation in body size, coloration and malformation incidence. Nevertheless, establishing direct links between phenotypic patterns and specific genetic mechanisms will require carefully controlled experimental frameworks.

Future studies should also expand the range of evaluated traits to include variables directly relevant to industrial insect production, such as feed conversion efficiency, survival, reproductive performance and tolerance to environmental or microbial stressors. In this context, host–microbiome interactions represent a promising field of research, as microbial communities may contribute to variation in host performance and developmental outcomes.

A multidisciplinary approach integrating phenotypic screening, controlled breeding designs and emerging molecular tools will be essential for the development of robust and efficient breeding strategies for *T. molitor* under large-scale production systems.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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