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Short communication: Substantial heritability of larval size in the black soldier fly reveals potential for selective breeding

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ABSTRACT

An underutilised tool to optimise the production of black soldier fly larvae (*Hermetia illucens* L.; Diptera: Stratiomyidae) is selective breeding. We validated that larval size is moderately heritable and should, therefore, respond to selection. We tested this premise by estimating additive genetic variation for larval size assessed as individual larval surface area (**ISA**), group surface area (**GSA**), and group weight (**GW**). A full-/half-sib design was used, where one virgin male fly was offered the opportunity to mate four virgin females. Each male had between one and three females that produced larvae, with a mean of 1.85 females per male. For each female that produced larvae, two cups with feed were prepared and fifty larvae were transferred to each cup. On day twelve after egg hatching, thirty larvae (full sibs) from each cup were randomly selected, and ISA was recorded (9 486 larvae from 92 sires and 169 dams). The GW of the thirty larvae was then recorded, and the GSA was calculated from the ISA of the thirty larvae in the group (317 full-sib groups). The data were analysed using sire-dam models including population average and batch as fixed effects and sire, dam and cup as random effects. The results showed moderate heritability for ISA (0.40), with a moderate effect of the common environment (0.21). For GSA and GW, moderate heritabilities were observed (0.39 and 0.30). These results show that there is great potential for black soldier fly breeders to implement selection for bigger larval size in their breeding programmes.

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Implications

Insect protein may be a sustainable food source for the growing world population. The larvae of the black soldier fly are among the most efficient converters of waste products into valuable protein. An underutilised tool to optimise the production of these larvae is selective breeding. This study shows that there is a great potential to select for larger black soldier fly larvae. These results are valuable for insect breeders, as they support strategic decision –making, for example when selecting the best individuals for breeding.

Introduction

Selective breeding provides a powerful method to increase commercial production of black soldier fly (**BSF**; *Hermetia illucens* L.; Diptera: Stratiomyidae) by improving desirable traits. One challenge hindering the implementation of selective breeding in BSF is the lack of comprehensive knowledge on genetic parameters for important production traits (Hansen et al., 2024a). Genetic parameter estimation is essential for implementing selective breeding, as it allows breeders to make informed decisions that maximise genetic gains. Evidence from other insect species suggests that considerable genetic variation exists for larval size traits. For instance, studies on the yellow mealworm (*Tenebrio molitor*; Sellem et al., 2024) and the house fly (*Musca domestica*; Hansen et al., 2024b) report heritability estimates ranging from low (0.09–0.11 in the housefly), to moderate-to-high (0.10–0.44 in the yellow mealworm). In this study, we investigate the extent of heritability of larval size in BSF colonies reared under experimental conditions and in the contexts of different trait definitions including larval size defined as individual's surface area, the group's surface area and the group's weight. Preliminary results of this study have been presented in abstract form (Zaalberg et al., 2025).

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Material and methods

Experimental design

We estimated additive genetic variation for larval size in BSF by mating 92 males (sires) to 169 females (dams), resulting in 169 full-sib families. Data were collected in three experimental batches. The number of sires, dams and records for each batch can be found in Table 1. Each of the 169 full-sib families were divided into two replicate groups. A total of 9 486 larvae were assessed for larval size measured as larval surface area, group weight, and group surface area 12 days after hatching (approximately 30 larvae per replicate family group). Variance components were estimated by fitting sire-dam models to the three measures of larval size. Each sire mated with one through three dams with an average of 1.84 dams. Each dam was virgin upon mating and was mated to a single sire. The pedigree of the sires and dams was unknown, and they were assumed unrelated. An overview of the experimental design is shown in Fig. 1.

Rearing procedure

The BSF stock used across all the batches was sourced from the production population at Enorm Biofactory (Flemming, Denmark). The Enorm population was established in 2016 with flies from Hermetia Baruth GmbH (Baruth, Germany) and was kept at a breeding density of at least 100.000 individuals per generation. At the beginning of each batch, pupae from this population were sampled and placed into individual plastic vials (95:25 mm H:Ø). The vials were closed with a foam stopper and placed in a climate chamber at 28 ± 0.5 °C and 90 ± 10% relative humidity under constant darkness. Emergence of flies was recorded every 24 h, and emerged flies were maintained in their own individual vials in the climate chamber for another 48 h to mature. After maturation, the sex of each fly was determined by visual inspection. One virgin male and four virgin females were then placed in a transparent plastic container (18.5:18.5:12 cm L:W:H) using the procedure described in Jensen et al. (2024). Each plastic container was lined with a wet paper towel at the bottom to maintain high humidity and was sealed with a transparent lid with an 8 cm Ø hole covered with a fine mesh for ventilation. The flies were subsequently given 4 h to mate at 27 °C and 50% RH under LED lights (112.5 cm 50 W Philips LED*life* VerticalGrow120 full spectrum) placed 8 cm above the container lids. After the 4 h, individual females were moved to plastic vials (115:23.5 mm H:Ø) with coarse foam stoppers for egg laying and returned to the climate chamber. Egg-laying was monitored every 24 h for 7 days. When an egg clutch was observed, the coarse foam stopper containing the eggs and the female fly were carefully removed from the vial. Thereafter, the coarse foam stopper with eggs was placed back into the vial, which was sealed off with a fine foam stopper. Egg-hatching was monitored every 24 h. For each vial with hatched eggs, two transparent plastic cups (70:95 mm H:Ø) were prepared with 30 g chicken feed (Paco Start, Danish Agricultural Grocery Company, Fredericia, Denmark) and 60 mL water. Larval density was controlled by taking approximately 50 random neonates from the vial in which they hatched and placing them into each of the two cups. The cups were covered with a transparent lid with a 65 mm Ø hole covered with a fine mesh for ventilation and placed in the climate chamber for 12 days.

Recording larval size

Data on individual larval surface area (**ISA**), full-sib group surface area (**GSA**), and full-sib group weight (**GW**) were collected when the larvae were 12 days old. For each cup separately, the larvae were washed under running water and dried on paper towels. Three cups that accidentally contained > 90 larvae were excluded from the dataset. From each cup, thirty larvae were selected randomly, and the GW of these larvae was recorded. Subsequently, the same thirty larvae were allocated to a transparent thirty-well-plate. The well-plate with larvae was placed on a glass plate with a camera positioned 56 cm below the glass plate and a light above the well-plate. The ISA was measured as the mean larval surface area determined from a 60 s live image acquisition using the software EthoVision (base version of EthoVision XT 15.0.1418 with a custom JavaScript for size estimation, Noldus, Wageningen, The Netherlands). The GSA was then obtained by aggregating the value of the thirty ISA records of one full-sib group from the same cup. For more details on the setup and procedure used to determine the surface area of individual larvae, see Laursen et al. (2021). Summary statistics on the three larval size traits are presented in Table 1.

Data analysis

Additive genetic variance for larval size was estimated by fitting univariate Gaussian linear sire-dam models to the three larval size traits (ISA, GW and GSA), using the AI-REML algorithm implemented in the DMU package (Madsen and Jensen 2013). The sire-dam model fitted to ISA was:

$$y = Xb + Us + Vd + Wc + e$$

The sire-dam model fitted to GSA and GW was:

$$y = Xb + Us + Vd + e$$

where y is a vector of phenotypic records; b is the vector fixed effects, including the population average and the batch (three levels); s is a vector of random sire effects $N(0, I_s\sigma_s^2)$; d is a vector of random dam effects $N(0, I_d\sigma_d^2)$; c is a vector of random common environment effects modelled using the id of the cup $N(0, I_c\sigma_c^2)$; e is a vector of random residuals $N(0, I_e\sigma_e^2)$; X, U, V, and W are design matrices associating fixed and random effects to the observations; I_s, I_d, I_c, and I_e are identify matrices of proper order; and σ_s^2, σ_d^2, σ_c^2, and σ_e^2 are unknown variances associated with the random sire, dam, common-environmental, and residual effects.

The (narrow sense) heritability (h^2) of ISA was calculated as $h^2 = \frac{4\sigma_s^2}{\sigma_s^2+\sigma_d^2+\sigma_c^2+\sigma_e^2}$, and for GW and GSA as $h^2 = \frac{4\sigma_s^2}{\sigma_s^2+\sigma_d^2+\sqrt{n}*\sigma_e^2}$, where

Table 1
Descriptive statistics for three black soldier fly larval size traits – individual surface area (ISA), group surface area (GSA) and group weight (GW) – across three batches of data.

			Records			Mean			SD		
Batch	Sires	Dams	ISA	GSA	GW	ISA	GSA	GW	ISA	GSA	GW
1	28	44	2 520	84	84	112.3	33.6	7.75	20.2	4.4	1.41
2	35	65	3 540	118	118	118.9	35.7	8.08	25.7	2.5	0.86
3	29	60	3 450	115	115	123.6	36.9	8.60	15.8	2.8	0.77
Total	92	169	9 510	317	317	118.8	35.6	8.12	17.6	3.51	1.06

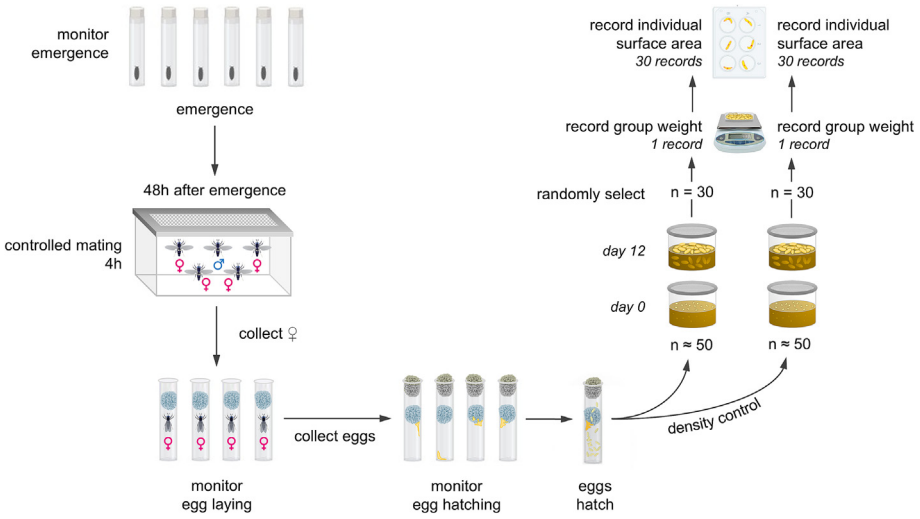

Fig. 1. Experimental design aimed at recording surface area of individual black soldier fly larvae and group weight of full-sib groups.

n (= 30) is the number of individuals in the full-sib groups. The proportion of variation explained by the common environment (c^2) for ISA was calculated as $c^2 = \frac{\sigma_c^2}{\sigma_s^2+\sigma_d^2+\sigma_c^2+\sigma_e^2}$. The asymptotic SEs for variance component estimates were obtained from the DMU-output, whereas the asymptotic SE of h^2 and c^2 were calculated using the delta method (Lynch and Walsh, 1998).

Results

Larval size was moderately heritable (Table 2). The heritability of ISA, GSA and GW was 0.40, 0.39, and 0.30, respectively. The proportion of the total phenotypic variance for ISA that was explained by the common environment was 0.21, which was approximately half the magnitude of the heritability for this trait (Table 2).

Boxplots for the three traits across the three batches are presented in Supplementary Fig. S1.

Discussion

Our findings demonstrate experimentally that larval size in BSF is moderately heritable, when assessed as ISA, GSA, and GW (0.30–0.40). These findings generally agree with the heritability estimates for larval size in the yellow mealworm (0.10–0.44; Sellem et al., 2024) and the housefly (0.09–0.11; Hansen et al., 2024b). The study of the housefly (Boatta et al., 2023; Hansen et al., 2024b) used the same method as the current study to analyse the individual surface area of housefly larvae. The results for the housefly suggested a remarkably lower heritability for individual surface area (0.09–0.11) than the moderate heritabilities (0.30–0.40) observed in our study. The heritabilities observed in the current study are encouraging for BSF-breeders because they indicate that larval size will be highly responsive to selective breeding and would make a worthy investment to increase the efficiency of BSF production.

The current study observed a moderate impact of the common environment (0.21) on the variation observed for larval size of BSF. In larvae of the housefly, the effect of the common environment was much stronger, as 50% of the observed variation in larval surface area was due to this factor (Hansen et al., 2024b). In the yellow mealworm, the effect of the common environment varied from 0.20 to 0.38. The lower impact of the common environment observed in the BSF and the yellow mealworm compared to the housefly was likely due to more constant rearing conditions in the BSF- and yellow mealworm studies, as well as the more constant larval density, and bigger rearing cups with larger group sizes (Hansen et al., 2024b). Common environmental effects can significantly impact selection decisions, making it essential to standardise conditions across families or lines to ensure accurate ranking of selection candidates. Variations in environmental conditions across families, lines, or populations may lead to genotype–by–environment interactions (Falconer and Mackay, 1996). If we do not adequately account for these genotype–by–environment interactions, this may result in incorrect ranking of candidates, which will diminish the effectiveness of selection

Finally, the current study observed sire variances that were much larger than the dam variances. This can indicate a limitation in our dataset. More follow-up studies will have to be conducted to understand why we observed differences in the sire- and dam-variance. Furthermore, the current study did not look at the genetic correlation of larval size to other important production traits, such as body composition. Generating more knowledge on the genetics of larval size will be an essential next step towards using larval size in breeding programmes using multitrait selection, with the aim to optimise BSF production. Finally, the breeding planning strategies and phenotyping methods used in this study are time–consuming,

Table 2
Heritability (h^2), common environmental effect (c^2), and variance components with SE for individual larval surface area (ISA), full-sib group surface area (GSA), and full-sib group weight (GW) in the black soldier fly. The presented variance components are the sire variance (σ_s^2), dam variance (σ_d^2), common environmental variance (σ_c^2) and residual variance (σ_e^2).

Trait	h^2	c^2	σ_s^2	σ_d^2 [1]	σ_c^2	σ_e^2
ISA (mm^2)	0.40 (0.11)	0.21 (0.02)	29.1 (9.9)	11.0 (8.6)	60.4 (7.7)	191.2 (2.8)
GSA (cm^2)	0.39 (0.12)	–	3.5 (1.1)	1.6 (0.8)	–	5.6 (0.6)
GW (g)	0.30 (0.10)	–	0.29 (0.10)	0.15 (0.09)	–	0.61 (0.07)

[1] For GSA and GW, the estimate of σ_d^2 includes both the genetic effect of the dam and the effect of the common environment.

and these are not suitable for large-scale breeding planning. To make selective breeding possible on a larger scale, the insect breeding process should be automated, for example, using computer-assisted phenotyping and sex determination of flies.

In conclusion, the current study shows that larval size is moderately heritable. More studies will be needed to better understand the genetics of BSF larval size, and how this trait can best be incorporated into BSF breeding programmes.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101534) can be found at the foot of the online page, in the Appendix section.

Ethics approval

Not applicable.

Data and model availability statement

Data are available upon reasonable request to the corresponding author. The data/models were not deposited in an official repository.

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

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

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Declaration of interest

Not applicable.

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