Received: 2 July 2024 | Accepted: 19 March 2025

DOI: 10.1111/eea.13565

Entomologia Experimentalis et Applicata

ORIGINAL ARTICLE

Special Issue: Trait Selection in Production Insects

Variation in strain performance and estimates of heritability of body size indicate considerable potential for genetic improvement of the black soldier fly (*Hermetia illucens*)

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Funding information
Biotechnology and Biological Sciences Research Council

Abstract

The black soldier fly (*Hermetia illucens* L., Diptera: Stratiomyidae) has emerged as a key species in the sustainable protein industry. Whilst genetic variation in performance has been suggested, the extent of heritability and genotype-by-environment (G × E) interactions in this species remains relatively unexplored. This study used a standardised split-brood design and linear mixed effect models to evaluate genetic effects at the between- and within-strain levels across diets as environments. In the first experiment, three strains were tested across three diets to identify strain × diet interactions. Significant interactions were observed for larval weights, development, family viability and protein content, indicating differential adaptability of strains to specific dietary substrates. The second experiment expanded on these results by testing two strains across three diets, but with a larger number of families ($n = 23$) and individual-level phenotyping of body size using 4018 individuals. Moreover, crude estimates of narrow-sense heritability (h^2) were obtained using a full-sibling design. Heritability estimates for larval and prepupal body size were high: $h^2 = 0.67$ and 0.78, respectively, although the estimates will be upwardly biased if there is substantial non-additive genetic variation. Together, our results highlight the potential for selective breeding to optimise black soldier fly strains for industrial applications, supporting the growth of this novel industry.

KEYWORDS

artificial selection, bioconversion, family-by-environment interactions, genotype-by-environment interactions, *Hermetia illucens*, life history, phenotypic variation, selective breeding, standing genetic diversity, sustainable protein source

INTRODUCTION

Using insects as food and feed has become a widely adopted approach to increasing sustainability in agriculture. Human population growth, projected to exceed 9 billion by 2050, necessitates an increase in food production, yet the current food system wastes one-third of total food annually, highlighting the need for a shift to sustainable methods (Alexandratos & Bruinsma, 2012; FAO, 2009; Food and Agriculture Organization of the United Nations, 2013; Gustavsson et al., 2011; Tilman et al., 2011; van Dijk et al., 2021). Insects require less land (Oonincx & de Boer, 2012), consume less water (Miglietta et al., 2015) and are considered to emit less greenhouse gases than traditional agricultural systems (Smetana et al., 2015, 2016). The black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), has become the focal species of the insect livestock industry (Athanassiou et al., 2024; Tomberlin & van Huis, 2020). The species has a remarkable ability to bioremediate a variety of organic wastes (Lalander et al., 2019; Newton et al., 2005;

Nguyen et al., 2015; Sheppard et al., 1994). Likely originating from South America, the black soldier fly has dispersed globally driven by anthropogenic expansion and globalisation (Kaya et al., 2021; Marshall et al., 2015). Intensive farming activity over the past two decades has also left genetic footprints of domestication in the genomes of farmed black soldier fly populations (Generalovic et al., 2021, 2023; Kaya et al., 2021). However, the large-scale application of genetic improvements within the insect industry is absent, in part due to a lack of fundamental understanding of genetic and phenotypic variation.

The ability of the black soldier fly to feed on a great variety of food and agricultural wastes (Surendra et al., 2020) has likely contributed to the successful globalisation of the species (Gligorescu et al., 2018; Hull et al., 2022; Rhode et al., 2020; Shumo et al., 2019). The ability may be due to phenotypic plasticity (West-Eberhard, 2008) but might also be due to genetic differences between strains. Plasticity may be adaptive and has been demonstrated to influence behaviour (Cunningham et al., 2019), morphology (Greene, 1989) and physiology (Brakefield et al., 1998) in several insect species. Genetic variation both among and within strains could also be utilised to optimise strains for specific industrial applications, such as feeding on different feedstocks. There is therefore a need for standardised common-garden experiments to differentiate between plastic and adaptive genotypic responses and quantify genotype-by-environment (G × E) interactions. Numerous studies have characterised performance, nutrition and waste conversion traits of the black soldier fly (Barragan-Fonseca et al., 2017; Spranghers et al., 2017; Surendra et al., 2020; Wang & Shelomi, 2017), but phenotypic variation across these studies is high (Gold et al., 2020), and the input substrate is sometimes poorly characterised (Barragan-Fonseca et al., 2021). In addition, the consequences of within-population genetic variation in the flies have been relatively unexplored.

A comparative study of three black soldier fly populations first explored larval performance and revealed considerable phenotypic diversity (Zhou et al., 2013), but without quantifying genetic differences. More recently, with increased genomic resources, studies have demonstrated considerable genetic diversity (Generalovic et al., 2021; Kaya et al., 2021; Zhan et al., 2019), including evidence for a cryptic species (Generalovic et al., 2023). One recent study was the first to combine genetic characterisation of strains with analysis of phenotypic diversity, demonstrating considerable differences in the feeding performance of genetically divergent strains (Sandrock et al., 2022). Strong G × E interactions in larval performance, nutritional profiles and emission traits highlight the potential adaptation of strains to particular diets (Sandrock et al., 2022). In addition, recent research documented the effect of black soldier fly genetic background on microbial communities impacting larval performance (Silvaraju et al., 2024). Traditional agriculture practices manage genetically differentiated stocks to optimise breeding and develop improvement programmes (Houston et al., 2020). However, this practice is largely absent from the insect livestock industry to date. Documenting both genetic and phenotypic diversity in wild and domesticated populations will reveal the potential for artificial selection to improve yields, generate specialised strains (Lee & Tollenaar, 2007) and promote economic efficiency as already occurs in more established agricultural species (Gjedrem et al., 2012). In order for selection to result in genetically differentiated populations (Gregory, 2009), selected traits must have a heritable component. Narrow-sense heritability (h^2), the proportion of phenotypic variance attributable to additive genetic variance, is vital in guiding breeding programmes and predicting the response to selection (Falconer, 1996; Holland et al., 2003). Successful genetic improvement therefore requires high levels of standing genetic diversity within populations due to family differences (Barton & Keightley, 2002). To our knowledge, however, no estimates of heritability have been made for black soldier fly performance traits with agricultural relevance.

Here, we examine the effects of phenotypic variation between strains and families of the black soldier fly for several economically relevant traits involved in performance, conversion ability and nutrition. First, we exposed three domesticated strains to three substrates (diets as environments) to explore differences between strain, diets, and to test for interactions between the two—a type of G × E interaction. Second, after observing high variation between strains, we estimated within-strain variance using a split-brood design and thousands of individual body size measurements. We used these experiments to address the following questions: (1) Do commercial strains respond differently to varying diets? (2) Do family-level genetic differences within strains also show such G × E? (3) What is the heritability of body size traits in the black soldier fly? (4) How do industrially relevant traits correlate with one another across environments?

MATERIALS AND METHODS

We carried out two common-garden rearing experiments, using families of three strains (Experiment 1) or two strains (Experiment 2), each reared on three different diets. We used this design to evaluate the impact of variation in domesticated strains of black soldier fly in response to changes in the environment. These data were used to investigate the extent of family-specific variation and heritability of economically relevant life-history traits. Figures (graphical abstract and Figure 1) were generated using Biorender.

Insect stock maintenance

Strain EVE was established as detailed in Generalovic et al. (2021), Industry-strain-A and Industry-strain-C were

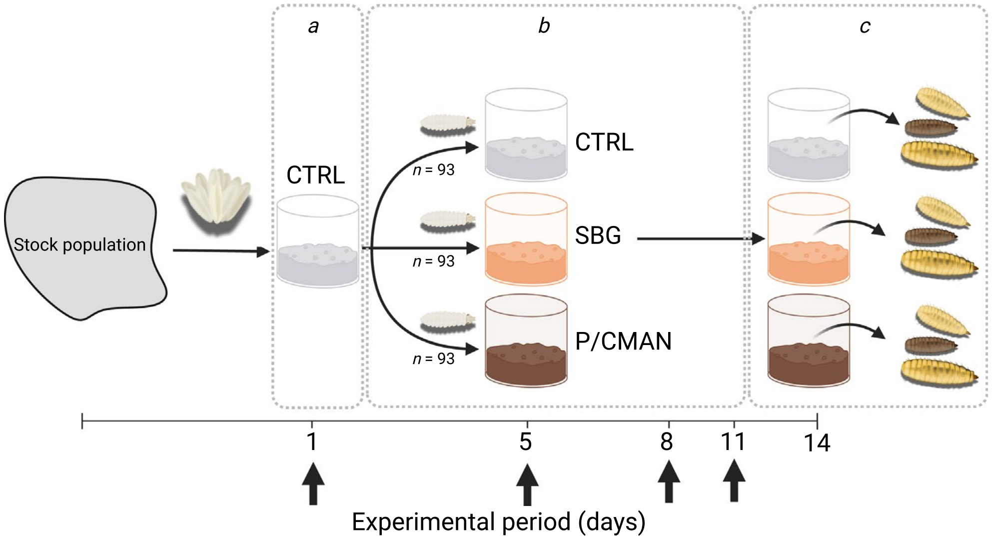


FIGURE 1 Phenotyping pipeline summary performed for both experiments within this study. In short, individual families of black solider fly (*Hermetia illucens*) were collected as independently laid egg clutches, by observation, from the stock population cages and isolated into rearing chambers containing control (CTRL) diet for five days (*a*). On the fifth day, individual larvae were removed, cleaned and weighed into aliquots of 93 larvae. Each family was split across three treatment chambers at standard densities each containing a different diet (*b*). Substrates included the control (Gainesville; CTRL), spent brewing grain (SBG) and a manure diet, poultry (PMAN) in Experiment 1 and cattle (CMAN) in Experiment 2. After the rearing phase ended split families were removed from the treatment chambers cleaned, re-weighed and counted at which point phenotypes were recorded (*c*). This method was performed for each strain used in Experiments 1 and 2.

obtained in May and July of 2019, respectively, and maintained at the University of Cambridge. Both Industry-strain-A, of Chinese origin, and Industry-strain-C, of Singaporean origin, were supplied by Better Origin (Entomics Biosystems Ltd.) originally purchased from independent commercial suppliers. No information is available on rearing conditions prior to strain sourcing. Phylogenetic analysis has previously identified all three strains, EVE, Industry-strain-A and Industry-strain-C, as belonging to lineage-*a* of the black soldier fly phylogeny (Generalovic et al., 2023). This lineage also harbours many of the domesticated populations seemingly originating from a wild North American source which appears to dominate the industry landscape (Generalovic et al., 2023; Kaya et al., 2021).

Larval stocks were maintained in controlled environments (CE) under a 16:8-h light: dark cycle, 29 ± 0.8°C, and 60% relative humidity unless otherwise stated. Larval density was maintained at an approximate 0.5 larva/cm^2, 5000 larvae per Euronorm rearing tray (20H*40W*60L cm), vertically stacked with airflow between trays, maintained at an approximate total population size of 5000–35 000 individuals every generation. Larval feeding was provided ad libitum on a custom diet consisting of 31.4% barley (Burnhills, UK), 5.02% wheat bran (Burnhills, UK), 0.38% dried brewing yeast (Natures Grub Ltd., UK), 0.38% egg yolk powder (Target Feeds, UK), 0.02% multivitamin powder (Bulk Powders, UK), 0.001% silage mix (Provita, Ireland) containing *Pediococcus pentosaceus* (Mees), *Lactobacillus plantarum* (Orla-Jensen) and *Lactobacillus brevis* (Orla-Jensen), mixed with 62.8% non-sterile water. In August 2020, the custom diet changed, reducing barley inclusion to 15.7% and supplemented with 15.7% spent brewing grain (Calvary's, UK). Nursery stages were maintained for 5 days at uncorrected densities using 1 kg of the outlined diet, followed by density correction on Day 5. Adult breeding cycles were maintained in a controlled environment greenhouse with approximate temperatures of 28°C and 80% humidity using natural light conditions. If natural light levels dropped below 32.8 k lux over the photo period of 06:00–18:00, an artificial light emitting diode (LED) (Eco Conversion Systems, US) would activate. Adults were housed within 90H*60W*60L cm cages (Qiansha, China) approximately 0.5 m beneath breeding lamps. Strains were maintained in a common-garden nursery for over 1 year under these detailed environmental conditions.

Phenotyping pipeline

Both experiments were performed using the same core phenotyping pipeline as follows (Figure 1). Set-up and nursery phase. As multiple matings have been previously observed within black soldier fly (Hoffmann et al., 2021), we harvested eggs from newly established breeding stock cages over a 30-min observation period to ensure families were unique to a single female. Whilst multiple matings were not recorded in these observation periods, it cannot be ruled out entirely that clutches may include multiple paternity due to female flies' ability to store sperm (Munsch-Masset et al., 2023). However, there is also evidence of ejaculate digestion in female black soldier fly adults (Manas et al., 2024). As such, to analyse this experiment, we assumed that each family contained only full sibs (see discussion below). Egg traps were made from four sheets of corrugated correx (0.3H*4W*8L cm) with vertical flute openings and placed over an oviposition site, a 1.9-L Tupperware container (Sistema, New Zealand), with a custom ventilated (6 cm diameter mesh) lid, containing ca. 500 g of one-week-old stock feed and used to collect clutches belonging to families. Clutches were weighed and set up in hatching containers (custom 1.9-L Tupperware container) for 3 days, containing an excess of control (CTRL) Gainesville substrate (36.6 g dry matter). Excess feed per individual was provided due to the non-standardised density of neonate hatched larva within each experimental container. Experimental containers were randomly placed within Euronorm trays to account for any CE derived environmental variation throughout the experiment. This initial nursery phase was maintained for 5 days (after a three-day hatching period) to rear families in isolation and standardise gut microbiota communities prior to the experiment phase due to a potential interaction between strain and microbe diversity (Greenwood et al., 2021).

Treatment phase

After the nursery period, larvae were removed from family-specific hatching containers and separated from frass (undigested substrate and insect excrement mixture). A split-brood design was performed for both experiments by counting three aliquots of 93 larvae, approximately homogenous in size, from each family, weighed as a group and added to an experimental container to ensure a fixed starting density of 1.2 larvae/cm^2 (Parra Paz et al., 2015). Each container was provided with 26 g dried mass of each respective experiment-specific substrate tested. Feeding was performed every 3 days to maintain a feeding schedule of 93 mg/larva/day wet mass as outlined in Parra Paz et al. (2015) for the remainder of the treatment phase. Trays containing experimental containers were rotated vertically after every feeding period. All substrates were prepared or collected within a week before use, stored at 4°C and manually mixed prior to each experiment. Moisture contents of treatments (Table S1) were adjusted to ca. 70% to further reduce experimental variation (Cammack & Tomberlin, 2017).

Collection phase

On Day 14 of the experiment, larvae were removed from experimental containers, separated from frass material, cleaned and weighed. Wet frass was weighed, and an approximate sample of 5 g was taken from every replicate and oven dried at 70°C for 24 h to obtain frass dried weights.

Experiment 1

Experiment 1 was performed in July 2020 using the core methodology outlined under 'Phenotyping pipeline'. The goal of the first experiment was to assess the effects of strain under controlled environmental conditions and to detect G × E interactions between strain and diet within the maintained stock populations. Therefore, we screened strains EVE, Industry-strain-A and Industry-strain-C using six families per strain split across three diets. Strains EVE, Industry-strain-A and Industry-strain-C were at Generations 20, 4 and 2, respectively since establishing as a stock population at the University of Cambridge. Family six from the EVE strain in the poultry manure diet was removed due to a predicted nematode infection (unconfirmed). Substrates represented a sample of the diverse commercial wastes that may be used under industry conditions to highlight the potential of tailored applications for the black soldier fly. Diets were varied across a control, consisting of Gainesville (CTRL), spent brewing grain (SBG; Calvary's brewery, Cambridge, UK) and decomposing poultry manure (PMAN; Wood farm, Cambridge, UK). Gainesville was used as it has been proposed as a suitable reference control diet for black soldier fly academic studies due to its common availability (Bosch et al., 2020; Hogsette, 1992).

Experiment 2

Experiment 2 was performed in March 2021 also using the core methodology outlined under 'Phenotyping pipeline' but with changes now described. To better characterise within-strain variance, we increased the number of families screened, whilst reducing the number of strains to two. In particular, strain Industry-strain-C was removed due to the similarity of its genetic profile to Industry-strain-A (Generalovic et al., 2023). Industry-strain-A was also closest in generation time to EVE, thereby helping to minimise between-strain differences in inbreeding. Strains EVE and Industry-strain-A were collected at Generations 27 and 11, post-establishment in our laboratory conditions, respectively. We collected 12 families from each strain (24 families in total); however, family six of the EVE strain did not yield enough neonate offspring to perform split families and so was removed, leaving in 23 families. Both a CTRL and SBG diet were used as in Experiment 1. SBG became a familiar substrate for Experiment 2 due to its inclusion into the stock feed in August 2020 as described above ('Insect stock maintenance'). In addition, fresh cattle manure (CMAN; Park farm, Abergavenny, UK) was used as a third diet due to availability. Protein samples were obtained for a total of six families. All substrates were stored and prepared under the conditions described above (Table S1).

Life-history traits

We collected data for life-history traits associated with economic value in the black soldier fly. These included performance, conversion ability and nutritional traits (Bosch et al., 2020). Performance traits included live harvest weights, batch development and family viability. Conversion efficiencies were represented by waste reduction rates. We used crude protein content as a measure for nutritional content. All weight-based measures were collected using a precision balance (AE 163, Mettler Toledo, UK).

Performance analysis

Live harvest weight (mg) was recorded as the mean individual larval weight (mg) on a wet-matter basis, derived from the total surviving grouped family weight divided by total surviving individuals:

$$\text{Live harvest weight (mg)} = \frac{\text{Total larval biomass at harvest (mg)}}{\text{No. of live individuals at end of experiment}}$$

Development was recorded as the number of individuals for each split-family reaching the prepupal stage by the end of the experiment, normalised by the total number of surviving individuals. A higher development value (%) indicates a family contained a higher number of prepupae and therefore faster life stage development. This metric is used in industry but can be confounded with survival. This method of measuring development is suited to black soldier fly due to prepupae being unfavourable (reduced size and suboptimal nutritional content) for commercial application (Liu et al., 2017):

$$\text{Development}\ (\%) = \frac{\text{No. of prepupae}}{\text{Total live individuals}} \times 100$$

Family viability, a measure of survival, was measured as the proportion of individuals who survived until the end of the experiment as follows:

$$\text{Family viability}\ (\%) = \frac{\text{No. of live individuals at end of experiment}}{\text{No. of individuals seeded at start of experiment}} \times 100$$

Conversion analysis

Waste reduction rates were generated from collecting residual treatment substrate and drying to obtain dry mass remaining from the total dry mass provided, and presented as a proportion:

$$\text{Waste Reduction Rate}\ (\%) = \frac{(\text{Total diet provided (mg)} - \text{Remaining feed residue (mg)})}{\text{Total diet provided (mg)}} \times 100$$

Nutrient analysis

A sample of six larvae per split-brood was freeze-dried, pooled and ground using a mortar and pestle for carbon-nitrogen-hydrogen (CNH) combustion analysis (CE-440 Elemental Analyzer, Exeter Analytical). Proximate protein content was determined using recovered nitrogen values multiplied by a species-specific conversion factor of 4.76 (Janssen et al., 2017) and presented as mean protein content (%) of each representative family.

Image analysis

Within Experiment 2, we predicted variation within and between families of two strains using larval and prepupal measurements of area (cm^2) as a proxy for size (i.e. weight), hereafter referred to as body size. Individuals were classified into life stages qualitatively using the darkened phenotype of prepupae as the key marker, and remaining individuals were deemed late-stage larvae (fifth larval stage). Prepupae and larvae were analysed independently due to the discrete smaller size resulting from the non-feeding period in prepupae. Individuals were immobilised by freezing at −20°C, and body size was obtained by recording area (cm^2) using a custom script (Montejo-Kovacevich et al., 2019) in ImageJ software (Rasband, 2011). Image analysis recorded the outline of individuals from a dorsal view as outlined in Montejo-Kovacevich et al. (2019) and recorded area (cm^2) for each individual when spaced apart in a batch. An internal scale was provided for calibration, and each image was calibrated independently. This method was also validated to confirm area as an adequate proxy for size by comparing weighed groups of black soldier fly pupae with area data captured using the image analysis pipeline. Pupae from an alternative stock population were used due to availability and the immobile nature of the life stage aiding ease of handling. Within this study, weight and area correlations produced values up to $r = 0.6$, adding confidence to the method. In addition, previous studies have also successfully utilised image-based phenotyping for black soldier fly (Laursen et al., 2021). Images of each family-by-life stage were internally labelled and captured using a smartphone camera (Samsung S9/S10) and manually quality controlled for artefacts.

Statistical analysis

To test for interactions between genotypes and diet, we used linear mixed effect models (LMM) as implemented in the lmerTest v3.1 package (Kuznetsova et al., 2017) in R v.2024.04.2 (RStudio Team, 2019). Plotting was performed using ggplot2 v3.5.1 (Wickham, 2016). Models generally took the following form:

$$y_{ijkl} = \mu + S_i + D_j + S \times D_{ij} + F_k(i) + \varepsilon_{ijkl}$$

where y_{ijkl} is the mean phenotypic measurement; μ is the overall mean; S_i, D_j are the fixed effects of strain and diet, $S \times D_{ij}$ is the strain–diet interaction; $F_k(i)$ is a random effect of the family nested within strain, and ε_{ijkl} is the residual error.

We also analysed strain-specific larval and prepupal size data, for the strains EVE and Industry-strain-A separately, using the model;

$$y_{ijkl} = \mu + D_j + F_k \times D_j + \varepsilon_{jk}$$

In all cases, statistically significant fixed effects were subsequently followed up with Tukey's honest significant difference (HSD) test using the emmeans v1.5.4 R package (Lenth, 2021). Model residual normality and heteroscedasticity assumptions were assessed using Shapiro–Wilk and Levene's tests. These assumptions were met for live harvest weight (arcsine square root transformed in Experiment 1 only), development and family viability (arcsine square root transformed in Experiment 2 only), but not for the other traits. These traits were therefore reanalysed using generalised linear mixed models as implemented in the packages lme4 v1.1 (Bates et al., 2015) and glmmTMB v1.1.10 (Brooks et al., 2017). In particular, development and family viability used the binomial (logit) function in Experiment 1, whilst waste reduction and protein content used a beta (logit) function in both experiments.

To obtain an estimate of the heritability, we fit the simpler model;

$$y_k = \mu + F_k + \varepsilon_k$$

after excluding any split-brood with fewer than 30 individuals. With our single-generation full-sib data, we estimated heritability from;

$$h^2 = \frac{2\sigma_F^2}{\sigma_P^2}$$

Falconer & Mackey (1996), where σ_P^2 is the total phenotypic variance and σ_F^2 is the within-family variance, which is equivalent to the intraclass correlation. We note that this is a crude estimate, because the within-family variance contains contributions from non-additive genetic variance (such as dominance and epistatic variance), which will upwardly bias our estimates. However, such contributions are expected to small for many populations (Hill et al., 2008). In addition, our estimate would be downwardly biased if our data contained any half-sibs, as the result of unobserved multiple matings by the female flies (see Section 2).

Finally, to test for correlations across industrially relevant traits, we used Pearson's Correlation coefficients (*r*). These correlations were visualised using the package corrplot v0.92 (Wei & Simko, 2021).

RESULTS

We performed two experiments to document intraspecific variation in performance, conversion ability and nutritional life-history traits of economic importance within the black soldier fly. In the first experiment, we used a total of 18 families belonging to three black soldier fly strains ($n = 6$ each) split across three diets. Average clutch sizes and standard deviations for strains EVE, Industry-strain-A and Industry-strain-C were 19.9 ± 8.9 mg, 21.5 ± 6.1 mg and 17.2 ± 4.3 mg, respectively. Using 5022 individuals split across families and diets, we quantified average family measurements for five phenotypes of interest. In the second experiment, we assessed mean split-family values of two strains and increased the number of families to 23. Clutch sizes and standard deviations for EVE and Industry-strain-A were 15.8 ± 5.9 mg and 12.3 ± 5.3 mg, respectively. We further took individual body size measurements as a proxy for fitness in both fifth instar larval ($n = 1237$) and prepupal ($n = 2781$) life stages, totalling 4,018 measurements. Our image analysis pipeline was validated using a set of black soldier fly pupae of known weight ($n = 66$). These data resulted in successful validation and an average correlation value between weight and area (cm^2) of $R = 0.9$, suggesting that size is an adequate proxy for weight (Figure S1).

Genetic differences and genotype-by-environment interactions shape phenotypic responses to diet in domesticated black soldier fly strains

The first experiment aimed to quantify the effects of environmental and genetic variability between strains of black soldier fly. We generated several estimates of family-level fitness, such as performance (harvest weights, survivability and development), conversion ability (waste reduction) and nutritional (protein) content (Table 1).

Diet and strain appear to influence several economically relevant life-history traits within our sampled strains. Substantial variation was explained by diet effects which strongly influenced most measured traits. This was expected when using a diverse array of substrates that vary in nutrient content and physical properties. In general, growth traits were lower on manures with reduced nutritional content as compared to controls. For example, larval weights, development and family viability were all high in the control diet and declined through to poultry manure (Figure 2). On average, weight-based measures were higher in the EVE strain when feeding on the control and spent brewing grain diets but not on poultry manure (Figure 2A). Recorded live harvest weights showed significant strain effects (linear mixed effects model (LMM): $F_{2,15} = 5.1$; $p = 0.02$). In addition, a strong strain×diet interaction was identified (LMM: $F_{4,30} = 25.6$; $p = 2.8 \times 10^{-9}$; Table 1). In particular, whereas the EVE strain was generally larger, both industry strains, particularly Industry-strain-A, developed faster on the control and spent brewing grain diets but not the poultry manure (Figure 2B). Whilst no strain effect was detected for development, a strong strain×diet interaction (GLMM $X^2_4 = 20.6$; $p < 0.001$) explained this observation. Family viability, a measure of group survival, over the treatment phase also indicated both strain (GLMM: $X^2_2 = 12.0$; $p = 0.002$) and strain×diet ($X^2_4 = 19.5$; $p = 0.001$) effects

TABLE 1 Summary and statistical analysis of life-history traits in domesticated strains of black soldier fly (*Hermetia illucens*) from Experiments 1 and 2.

							Control (CTRL)			**Spent brewing grains (SBG)**			**Manure (P/C MAN)**		
							Strain								
Life-history trait	**Experiment**	***n***	**Test**	**Diet**	**Strain**	**G×E**	**EVE**	**Industry-strain-A**	**Industry-strain-C**	**EVE**	**Industry-strain-A**	**Industry-strain-C**	**EVE**	**Industry-strain-A**	**Industry-strain-C**
Live harvest weight (mg)	01	53	*F*	5480.2***	5.1*	25.7***	152.5^{e}	148de	143.3^{cde}	139.7^{cd}	138.5^{cd}	135.5^{c}	28.6^{a}	38.5^{b}	41.6^{b}
	02	69	*F*	142.2***	3.4●	4.6*	164.8^{d}	162.4^{cd}	NA	102.3^{a}	99.4^{a}	NA	148.0^{c}	125.7^{b}	NA
Development Rate (%)	01	53	X^2	338.2***	1.3	20.6***	96.4^{d}	97.4^{d}	97.7^{d}	44.2^{b}	74.6^{c}	68.6^{c}	1.9^{a}	0.6^{a}	1.2^{a}
	02	69	*F*	161.7***	17.4***	1.7	95.6^{c}	98.5^{c}	NA	49.5^{ab}	65.4^{b}	NA	31.4^{a}	55.8^{b}	NA
Family viability (%)	01	53	X^2	48.6***	12.0**	19.5***	99.3^{c}	96.8^{bc}	99.3^{c}	98.4^{c}	96.6^{bc}	98.2^{c}	89.7^{a}	93.7^{ab}	93.0^{ab}
	02	69	*F*	51.5***	0.3	1.7	95.1^{b}	95.2^{b}	NA	94.9^{b}	96.5^{b}	NA	85.0^{a}	80.6^{a}	NA
Waste reduction rate (dry matter; %)	01	53	X^2	573.4***	1.4	6.7	78.2^{c}	78.6^{c}	76.6^{c}	56.3^{b}	58.2^{b}	52.4^{b}	29.2^{a}	26.5^{a}	26.8^{a}
	02	69	X^2	33.1***	0.003	0.53	52.0^{bc}	51.8^{bc}	NA	43.8^{ab}	41.5^{a}	NA	62.7^{d}	59.1^{cd}	NA
Protein content (dry matter; %)	01	53	X^2	5.9●	0.9	4.4	39.4^{a}	38.7^{a}	37.5^{a}	36.5^{a}	36.1^{a}	40.4^{a}	34.0^{a}	33.6^{a}	34.2^{a}
	02	47	X^2	73.3***	9.1**	10.1***	31.9^{bc}	33.8^{d}	NA	32.9^{cd}	33.2^{cd}	NA	28.7^{a}	31.3^{b}	NA

Note: Strains EVE, Industry-strain-A and Industry-strain-C across Control (CTRL) and spent brewing grain (SBG) and poultry manure (PMAN) substrates were used for Experiment 1. Strains EVE and Industry-strain-A were used for Experiment 2 only with the same diets except for poultry manure which was switched for cattle manure (CMAN). Indication of significant fixed effects (*F*-values or *X2*) under 'Diet', 'Strain' and 'Genotype-by-Environment interactions (G×E)' are indicated as follows: ● $=p<0.1$, * $=p<0.05$, ** $=p<0.01$, *** $=p<0.001$. Square root data transformation was performed on live harvest weight of Experiment 1. Development and family viability data from Experiment 2 was arcsine transformed. Superscript notations provide post hoc test results for G×E interactions with groups with the same letter indicated no significant difference whilst different letters indicate significant differences at $\alpha=0.05$.

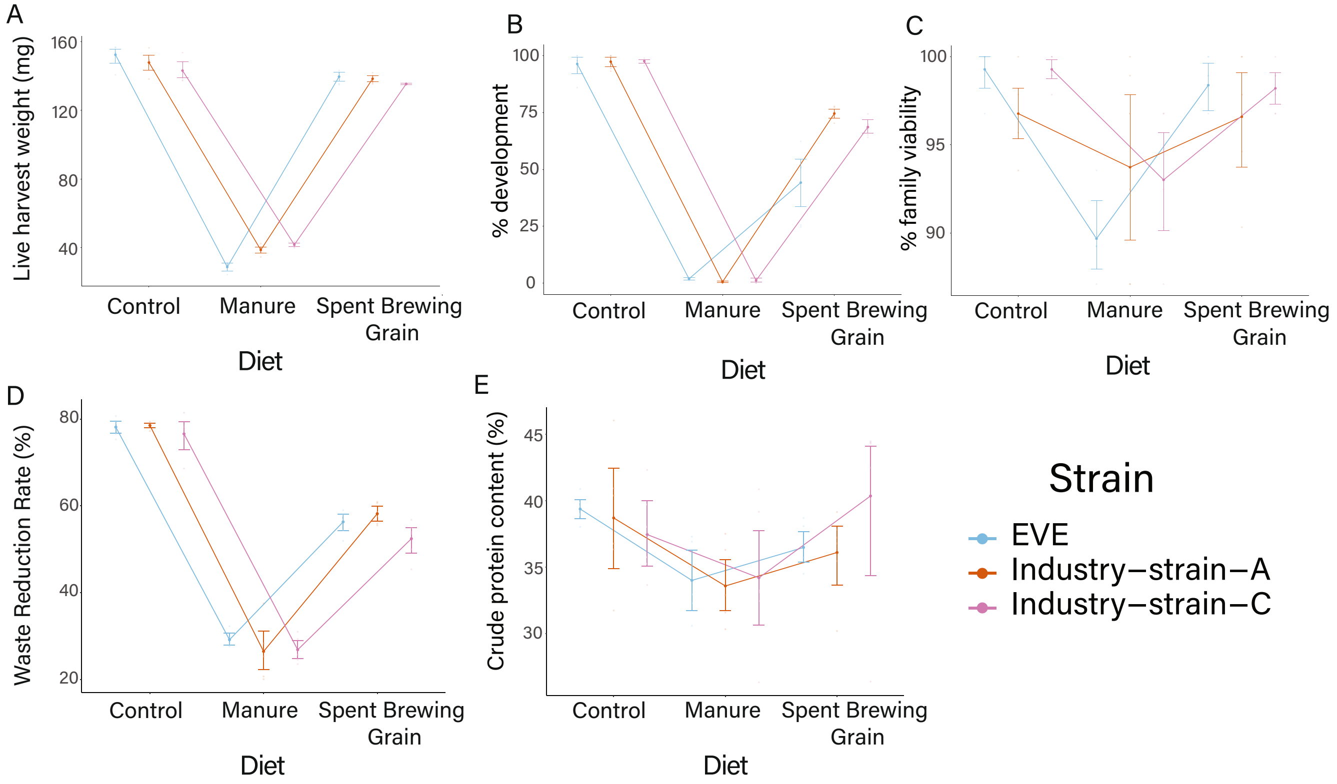


FIGURE 2 Reaction norms plotted for life-history traits of three domesticated black solider fly (*Hermetia illucens*) strains across three diets in Experiment 1. Diets included a control, spent brewing grain and poultry manure. Phenotypes of live harvest weight (mg; A), development (%; B), family viability, a measure of survival, (%; C), waste reduction rate (%; D) and crude protein content (%; E). Data highlight that dietary response is strain dependant for many traits, such as harvest weight (A) whereas less impactful for waste reduction rate (D). 95% confidence intervals are presented for each mean family trait value. Overall, reaction norms show the importance of both genetic and environmental contributions to trait variation in black soldier fly.

(Figure 2C). Conversion ability was inferred from the overall organic mass reduced from the experimental container (waste reduction rate). No effect of strain or interaction was found in the ability to reduce waste matter from the system in both experiments. However, Industry-strain-A appeared to show the highest waste reduction ability in the control and spent grain diets, whilst EVE outperformed all strains on manure-based diets (Figure 2D). Larval protein content showed no difference between strains across all diets but also only showed marginal significance towards the fixed effect of diet (GLMM: $X^2_2 = 5.9$, $p = 0.052$; Figure 2E). The prevalence of G × E interactions and non-parallel reaction norms also suggests that phenotypic plasticity differs among strains (Figure 2).

Additional support for both strain and strain×diet effects was also identified in Experiment 2 (Table 1; Figure S1). Larval weights showed a marginal impact of strain which tended towards significance (LMM: $F_{1,21} = 3.4$, $p = 0.081$), whereas a strain×diet interaction (LMM: $F_{2,42} = 4.6$, $p = 0.016$) was identified. No strain×diet interaction was found for development in the second experiment, but strain showed a strongly significant effect (LMM: $F_{1,21} = 17.4$, $p < 0.001$). Despite previously showing both a strain and strain×diet interaction for family viability, this was not observed in Experiment 2. Waste reduction also did not show any major effects other than diet (GLMM: $X^2_2 = 33.1$, $p = 6.4 \times 10^{-8}$). Protein content, unlike Experiment 1, identified a strain (GLMM: $X^2_1 = 9.1$; $p = 0.003$) and strain × diet effect (GLMM: $X^2_2 = 10.1$; $p = 0.006$). Body size data captured on individuals from Experiment 2 also provided support for significant effects of strain (LMM: $F_{1,34} = 30.4$, $p = 3.7 \times 10^{-6}$) and a strain×diet interaction (LMM: $F_{2,1290} = 30.3$, $p = 1.4 \times 10^{-13}$) for larval body size. Prepupal body size also showed small effects of strain (LMM: $F_{1,21} = 6.8$; $p = 0.012$) and large strain×diet interaction effects (LMM: $F_{2,2758} = 221.0$; $p = 2.0 \times 10^{-16}$). Full model outputs and effect sizes are presented in Table S2. These additional insights provide substantial support for G × E interactions and a strong genetic component to the phenotypic variation observed in black soldier fly.

Phenotypic variability is derived from family variation and shaped by family-by-environment interactions

In our second experiment, we reduced the number of strains to two and increased the number of families sampled ($n = 23$) to obtain greater insight into within-strain variation. Experiment 2 documented considerable phenotypic variance explained by family. Variation between families was a substantial proportion of the total phenotypic variance in live harvest weight, up to 36% (Table S2), whereas for family viability and development families, it explained just 14% each (Figure 3). Additional data for Experiment 2 were obtained by image analysis, capturing body size of both larval and prepupal life stages. On average, 26% and 33% of phenotypic variance was attributable to family effects in larval and prepupal body size, respectively (Figure 3; Table S2).

Whilst most of our measures were taken as family-level means, we also obtained individual-level measurements of body size. Within strains (analysed separately) we observed high levels of body size variation both within and between families, at both larval (Figure 4A,B) and prepupal (Figure 4C,D) life stages (Table S3). In general, families showed striking distributions across and within diets, particularly within Industry-strain-A where higher average body size was observed within the spent brewing grain diet with approximately half the families drastically differing on the control and manure diets (Figure 4D). Whilst diet explained just 15.3% of the variance in larval body size for the EVE strain (LM: $F_{2,747} = 110.30$, $p < 2.2 \times 10^{-16}$), only 0.6% of variance was explained by diet within Industry-strain-A (LM: $F_{2,501} = 3.97$, $p = 0.019$). Interestingly, family effects explained 21.4% (LM: $F_{10,747} = 30.89$, $p < 2.2 \times 10^{16}$) and 28.2% (LM: $F_{11,501} = 34.40$, $p < 2.0 \times 10^{-16}$) of variance within EVE and Industry-strain-A, respectively, whereas the family-by-environment interaction explained 11.6% for the EVE strain (LM: $F_{17,747} = 9.81$, $p < 2.2 \times 10^{-16}$) and up to 33.9% of variance within Industry-strain-A (LM: $F_{11,501} = 41.32$, $p < 2.0 \times 10^{-16}$). Summary output of models for body size variation is presented in Table S3.

Evidence for significant family effects in the prepupal stage was also seen, in strain EVE 50.3% of the variance was explained by diet (LM: $F_{2,1104} = 911.16$, $p < 2.0 \times 10^{-16}$) suggesting a stronger influence of diet on growth. Whereas for Industry-strain-A observed the equivalent figure was just 11.5% (LM: $F_{2,1608} = 378.56$, $p < 2.0 \times 10^{-16}$). Between-family variance was also strikingly different between strains, within EVE 9.9% of prepupal body size variance was explained by family effects (LM: $F_{10,1104} = 35.79$, $p < 2.0 \times 10^{-16}$) whilst the figure was 31.0% for Industry-strain-A (LM: $F_{11,1608} = 186.08$, $p < 2.0 \times 10^{-16}$). Additionally, family-by-environment interactions explained up to 33.2% of variance within Industry-strain-A (LM: $F_{22,1608} = 99.50$, $p < 2.0 \times 10^{-16}$) but just 9.3% in EVE (LM: $F_{20,1104} = 16.83$, $p < 2.0 \times 10^{-16}$). Residual variance was found to be much lower in the prepupal models compared with the larval models at 30.5% for EVE and 24.4% for Industry-strain-A. Full model outputs are presented in Table S3. Overall, this indicates that EVE families are effective generalists whilst Industry-strain-A shows potential for specialist roles, especially if included in a breeding programme.

Body size heritability indicates genetic potential for selective breeding in the black soldier fly

Given the significant family and individual-level variance observed, we next estimated the heritable component of body size, using individual data from Experiment 2 (Table 2). We estimated narrow-sense heritability (h^2) for both larval and prepupal body size. Using 1237 larval and 2782 prepupal body size phenotypes from 20 and 23 families respectively, we estimated $h^2 = 0.674 \pm 0.049$ for larval body size and $h^2 = 0.782 \pm 0.030$ for prepupal body size (Table 2). Given our full-sib design, these high estimates are likely to be inflated by non-additive components of the genetic variance; nonetheless, they indicate strong potential for selective breeding in this species.

Life-history traits show potential diet-specific trade-offs in the black soldier fly

Using mean family-level phenotypes from Experiment 2, we inferred the correlation matrix between the traits (Figure 5). Family phenotypes were averaged across strains as a representative of the genetic background to current domesticated populations as currently used throughout the industry (Generalovic et al., 2023; Kaya et al., 2021). We found significant correlations between several traits of economic importance (Figure 5). Most importantly, increased larval weight appears to have a negative relationship with development, suggesting a possible trade-off on both control (Pearson correlation test: $r = -0.47$, $p = 0.023$) and manure (Pearson correlation test: $r = -0.78$, $p = 1.2 \times 10^{-5}$) diets. An increased waste reduction ability is observed with larger larvae, indicating more efficient bioconversion in grain-based diets (control; Pearson correlation test: $r = 0.62$; $p = 0.002$), whereas the reduced waste reduction ability observed when reared on spent brewing grains appears to be due to faster development (Figure 2B) with the majority of families reaching the non-feeding prepupal stage more rapidly. These data also demonstrate that our image analysis pipeline is effective at generating representative size-based phenotype data, with harvest weights (mg) correlating up to $r = 0.64$ and $r = 0.56$ for larval size (cm^2) and prepupal size (cm^2) respectively.

DISCUSSION

Exploring phenotypic variation and identifying quantitative traits with a heritable genetic basis is an essential first step towards livestock improvement. Here, we quantify the genetic contribution to variation in traits of commercial importance, contributing to our understanding of the black soldier fly as a successful insect livestock species. We

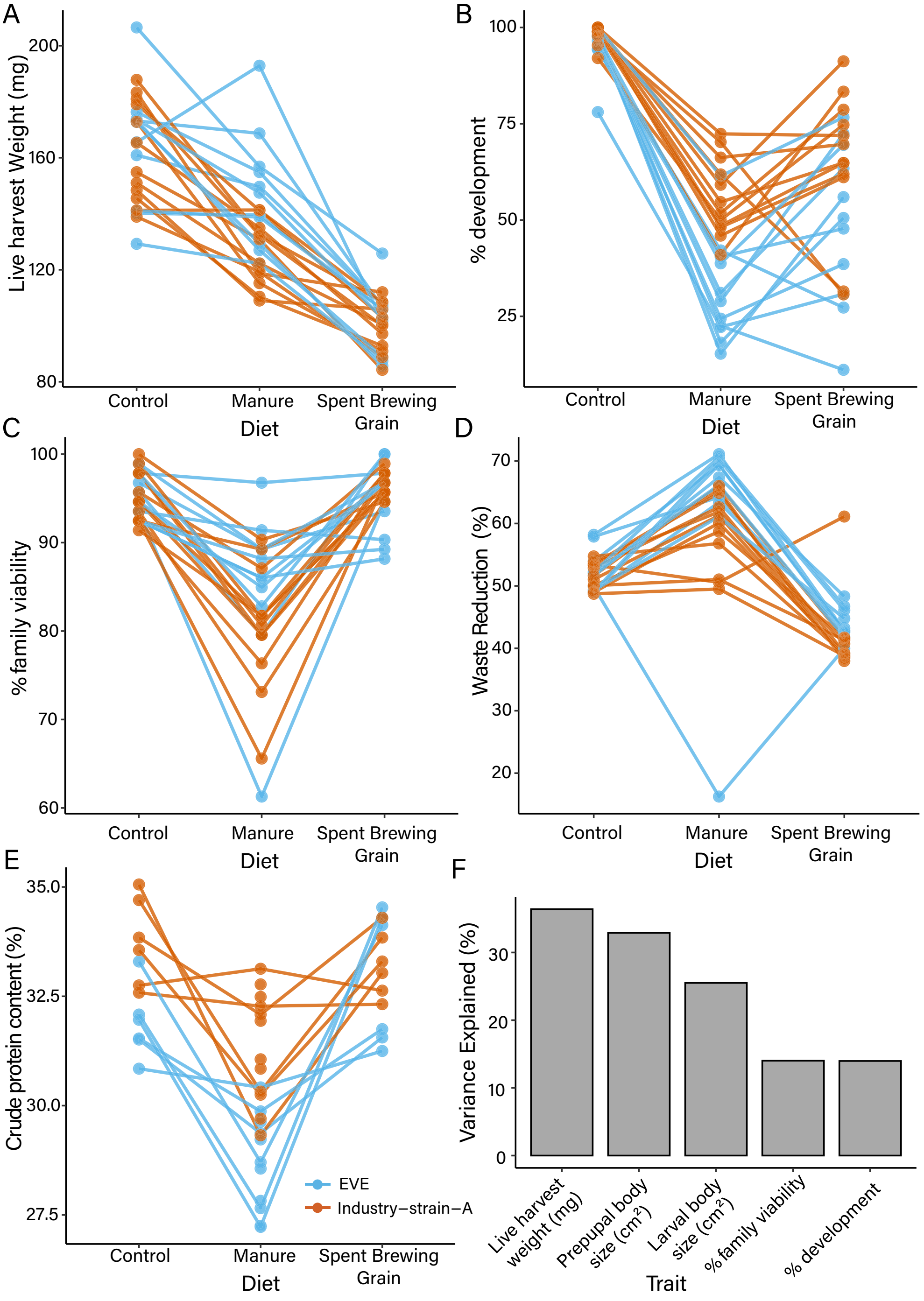


FIGURE 3 Split family-level life-history trait responses in black solider fly (*Hermetia illucens*) strains across diets from Experiment 2. Reaction norms highlight the highly varied response across environments both between and within strains and families represented by a mean and 95% confidence interval. Diets included a control, spent brewing grain and cattle manure. Phenotypes of live harvest weight (mg; A), development (%; B), family viability, a measure of survival, (%; C), waste reduction rate (%; D) and crude protein content (%; E) are shown including the model output of variation (%) explained by family from each trait (F). Data highlight that whilst strain choice is important for traits, such as harvest weight and protein content, family is just as strong a diver in phenotypic variability. Whereas, traits, such as waste reduction, are more predictable in their response on both the family and strain level.

first demonstrated that different strains show divergent phenotypes across a range of diets. Furthermore, family-level variance suggested high levels of genetic variation even within strains, which varied depending on strain. Despite extensive farming of domesticated strains and presumably associated inbreeding, family-level variation

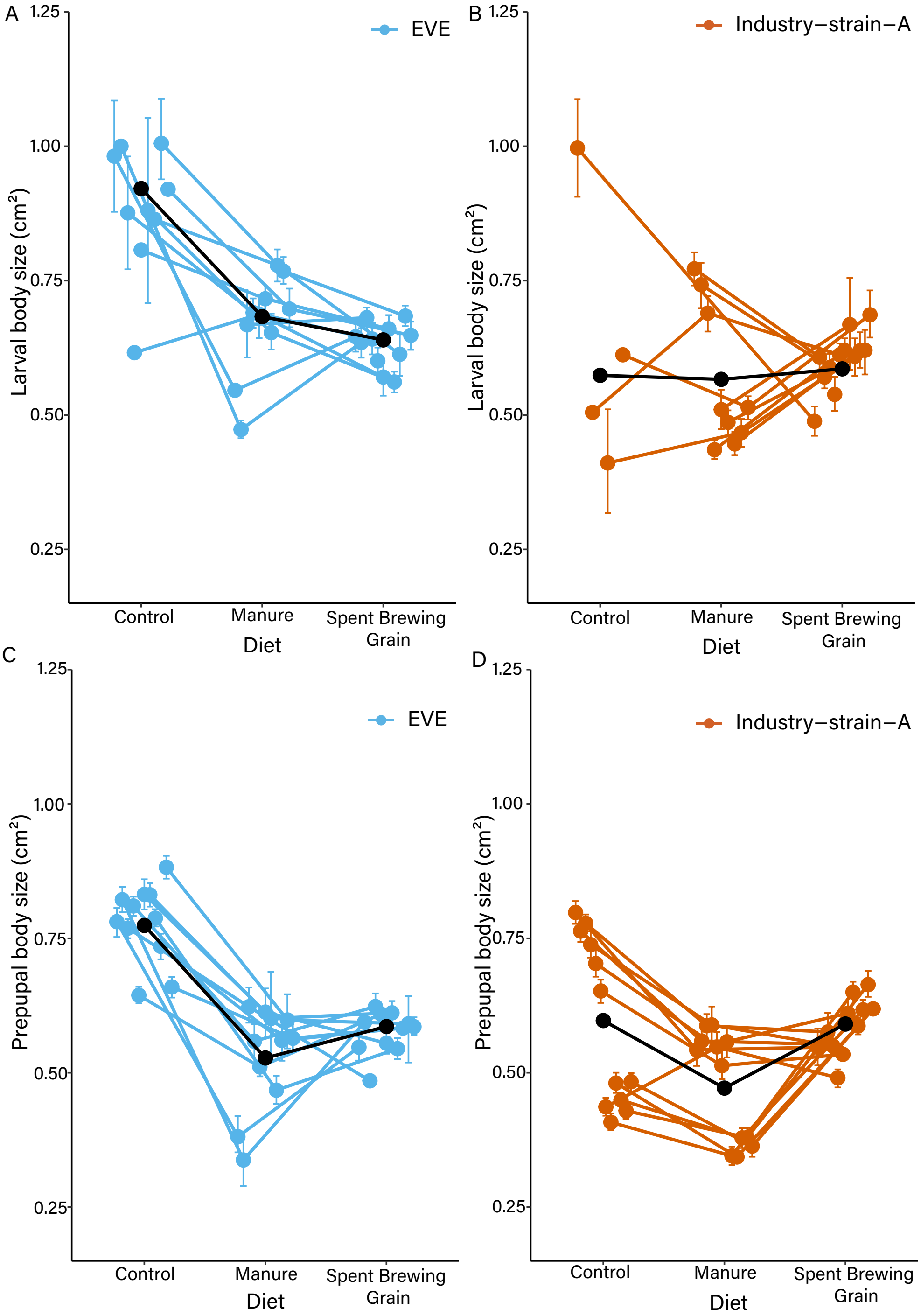


FIGURE 4 Family-by-environment responses of larval (A and B) and prepupal (C and D) body size for EVE and Industry-strain-A black solider fly (*Hermetia illucens*) strains across control, spent brewing grain and cattle manure diets in Experiment 2. Mean strain body size within each diet is indicated by a solid black reaction norm revealing substantial within strain and between-family variation whereas linked means across diets represent individual families and within-family variation. Whilst diet is a major driver for body size, strain EVE appears a stronger generalist whereas family-specific effects within Industry-strain-A highlights potential for specialist applications. 95% confidence intervals are presented for each mean family trait value. Overall, strong family-by-environment interactions presented here suggest that breeding strategies need to consider diet-specific performance for trait optimisation.

remains a substantial portion of variance in many traits, particularly in size and weight phenotypes. We use these data to provide the first estimate of heritability (h^2), the initial assessment of the genetic component required for selective breeding, for a key trait of commercial importance, body size, which highlights significant potential for the adoption of breeding programmes and genetic management.

TABLE 2 Estimates of narrow-sense heritability (h^2) for body size traits of the black soldier fly (*Hermetia illucens*) from Experiment 2.

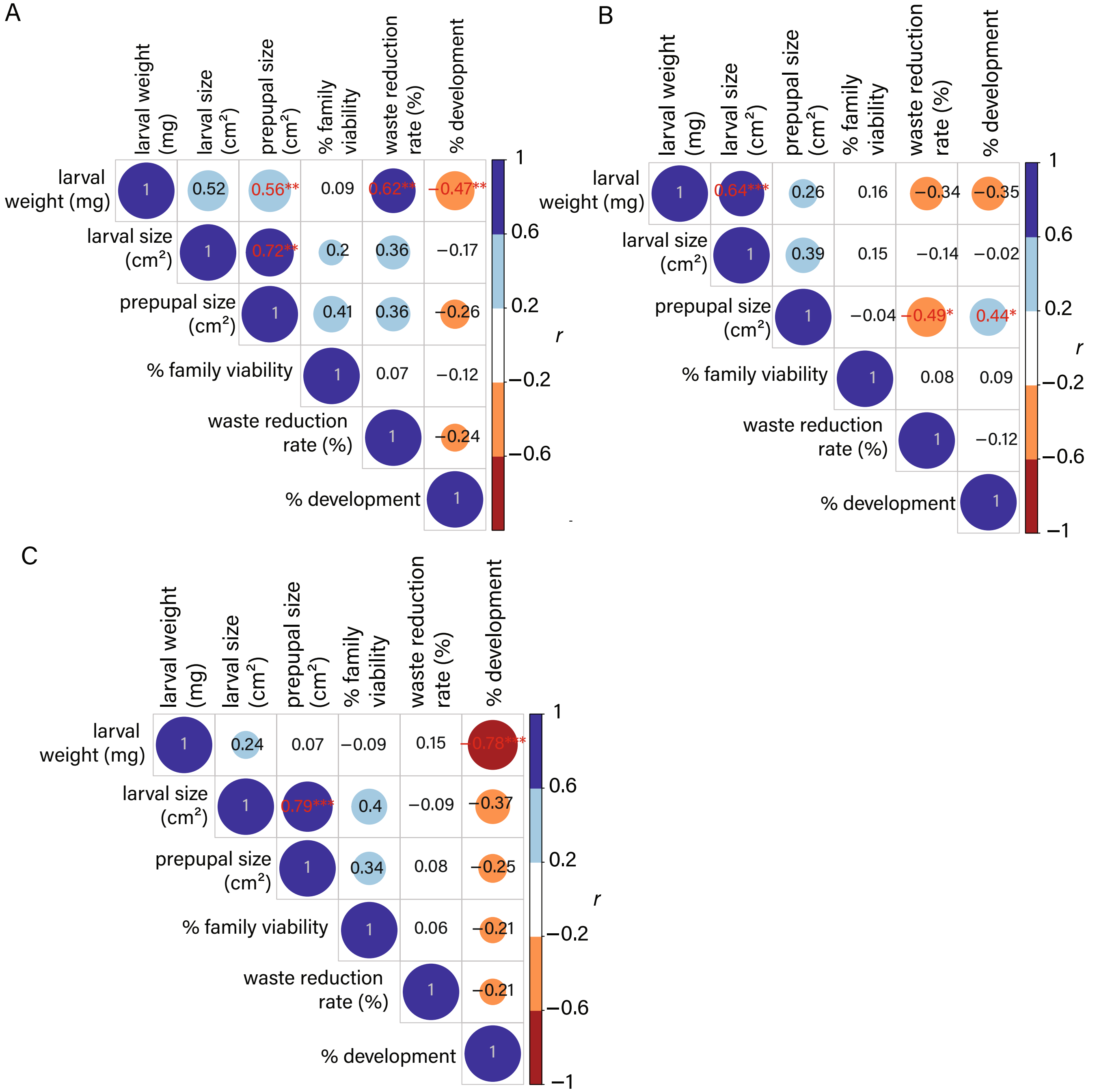
Trait	N (individuals)	N (families)	σ^2_G	σ^2_E	σ^2_P	h^2	Standard deviation (h^2)	95% confidence interval (h^2)
Larval body size	1237	20	0.006	0.012	0.175	0.674	0.049	0.577–0.767
Prepupal body size	2781	23	0.010	0.016	0.026	0.782	0.030	0.722–0.839

Note: Mean and standard deviation values are provided and h^2 estimates are averaged across all strains and diets as an approximate representation of the diverse commercial environment. Data show a high heritability for body size in both life stages indicating a genetic contribution to body size. All genetic (G), residual (E; environmental) and phenotypic (P) variances are provided, including the number of families and individuals screened post-removal of families with less than 30 individuals.

Genetic differences and genotype-by-environment interactions shape phenotypic responses in domesticated black soldier fly strains

Elucidating how organisms adapt to environmental variability is critical to understanding evolution. In a more applied context, characterising the genetic and phenotypic background of strains on various diets will provide the foundation for understanding plasticity and adaptation in the black soldier fly. The importance of plasticity and interaction with genetic effects in organisms' fitness is well studied (Moczek, 2010; Nylin & Gotthard, 1998; West-Eberhard, 1989; Whitman & Agrawal, 2009). Remarkable plasticity has been identified in the black soldier fly with the same strains being able to feed on a wide variety of organic substrates, and showing considerable variation in development time, size and other phenotypes (Zhou et al., 2013). However, there is also evidence for differences between strains in traits of economic importance, implying genetic differences (Sandrock et al., 2022; Zhou et al., 2013). Using previous phylogenetic data, the assessed strains in this study were all found within a single lineage (lineage-*a*). Industry-strain-A and Industry-strain-C are sister strains whilst EVE was found to be genetically more distant and differentiated (F_{ST} up to 0.12; Generalovic et al., 2023). Phenotypic patterns within our study mirrored these phylogenetic relationships, EVE appeared phenotypically divergent to both Industry-strain-A and -C (Generalovic et al., 2023). Within Sandrock et al. (2022), strains appear to be derived from both lineage-*a* and -*b* and therefore show greater genetic dissimilarity and differentiation (F_{ST} up to 0.33) explaining the stronger G×E effects observed and further supporting a genetic basis to phenotypic divergence. Given the extensive genetic diversity found globally and the phenotypic variation observed between closely related populations in this study, it is likely that numerous undocumented, genetically adapted populations may exist in the wild, potentially harbouring commercially valuable phenotypes (Generalovic et al., 2023; Guilliet et al., 2022; Kaya et al., 2021). This presents significant opportunities for advancing our understanding of evolutionary adaption in this recently globally dispersed insect and for harnessing its unique abilities to bioconvert specific organic waste streams.

Considerable genetic diversity occurs among global black soldier fly populations (Kaya et al., 2021). Ecological niches of wild populations likely reflect adaptation during range expansions and a current cosmopolitan distribution (Kaya et al., 2021; Marshall et al., 2015). In addition, populations maintained in agricultural conditions lack standardisation in rearing processes and climatic control (Bosch et al., 2020). Exposure to heterogeneous conditions in the wild and industry is therefore common. Interestingly, selection under heterogeneous environments can be hindered in the presence of G×E effects (Lazzaro et al., 2008). A decrease in genotype–phenotype correlation within populations due to the presence of G×E interactions may prevent selection from occurring and potentially drive the maintenance of polymorphisms (Lazzaro et al., 2008). In some species, such as stalk-eyed flies, *Cyrtodiopsis dalmanni*, sexual ornaments are genotype–environment-specific, causing variation in female mate choice and the maintenance of genetic variation among strains (David et al., 2000). The presence of even weak G×E effects between closely related populations, as used in this study, suggests they may play a role in the maintenance of phenotypic and genotypic diversity within black soldier fly (Turelli & Barton, 2004). However, there is also no evidence to date of reproductive incompatibilities between strains of black soldier fly, suggesting that diversity may also be maintained by hybridisation (Ståhls et al., 2020). Furthermore, substrates treated with black soldier fly larvae are usually comprised of decaying organic wastes and therefore contain a rich microbe diversity (De Smet et al., 2018). Recent evidence suggests that genetic background has a strong impact on microbe diversity (Greenwood et al., 2021), and further that black soldier fly transcription profiles appear to heavily depend on microbe-dependent co-expression (Auger et al., 2023). These host–microbe interactions may be confounding black soldier fly genetic effects. Populations of Drosophila inoculated with unique microbe communities showed varied reproduction, nutrition and behaviour phenotypes; however, when sterilised, phenotypic variation was absent (Téfit et al., 2022). Despite controlling the exogenous microbe community at the priming phase in both of our experiments, the introduction of novel microorganisms in the treatment phase of our study may have contributed to phenotypic diversity. Identifying optimal strain–microbe combinations across each diet may also provide an important strategy for improving commercial productivity. Ultimately, these data suggest that strain–diet choice is essential for effective black soldier fly applications.



A

	larval weight (mg)	larval size (cm^2)	prepupal size (cm^2)	% family viability	waste reduction rate (%)	% development
larval weight (mg)	1	0.52	0.56**	0.09	0.62**	−0.47**
larval size (cm^2)		1	0.72**	0.2	0.36	−0.17
prepupal size (cm^2)			1	0.41	0.36	−0.26
% family viability				1	0.07	−0.12
waste reduction rate (%)					1	−0.24
% development						1

B

	larval weight (mg)	larval size (cm^2)	prepupal size (cm^2)	% family viability	waste reduction rate (%)	% development
larval weight (mg)	1	0.64***	0.26	0.16	−0.34	−0.35
larval size (cm^2)		1	0.39	0.15	−0.14	−0.02
prepupal size (cm^2)			1	−0.04	−0.49*	0.44*
% family viability				1	0.08	0.09
waste reduction rate (%)					1	−0.12
% development						1

C

	larval weight (mg)	larval size (cm^2)	prepupal size (cm^2)	% family viability	waste reduction rate (%)	% development
larval weight (mg)	1	0.24	0.07	−0.09	0.15	−0.78***
larval size (cm^2)		1	0.79***	0.4	−0.09	−0.37
prepupal size (cm^2)			1	0.34	0.08	−0.25
% family viability				1	0.06	−0.21
waste reduction rate (%)					1	−0.21
% development						1

FIGURE 5 Life-history trait correlation matrix for domesticated black solider fly (*Hermetia illucens*) populations across various diets. Diets include control (Gainsville) (A), spent brewing grains (B) and cattle manure (C). Traits recorded from Experiment 2 are presented to show diet-specific trends and trade-offs. Notable patterns identified consistent positive correlation (blue) between larval weight and development indicating a trade-off. Whereas a negative correlation was found between size and weights and waste reduction rates. Significant values are indicated in red text and thresholds are set at $\alpha = 0.05$ and presented as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$. These data suggest diet-specific traits present within industrial black soldier fly populations.

Phenotypic variability includes between-family variation and family-by-environment interactions

Whereas recent research has explored the impact of environmental dynamics on the productivity of the black soldier fly, genetics has been largely overlooked. This study has demonstrated the impact of the genetic background of strain on performance traits in the black soldier fly. However, it appears that the effect of family is also considerable. Genetic improvement relies on quantitative traits being heritable across generations to accumulate genetic gains (Hill, 2010). Variation in quantitative traits is therefore required within and between families for successful genetic improvement. Previous studies have not explored the effect of family on phenotypes (Barragan-Fonseca et al., 2021; Sandrock et al., 2022; Zhou et al., 2013). The data presented here therefore provide the first evidence for family variation and strong family-by-environment interactions within strains for fitness-associated phenotypes. This suggests that genetic variation within strains and families has considerable potential for diet-specific adaptation. Families belonging to Industry-strain-A appear to show a somewhat bi-modal distribution in fitness across the manure diet, suggesting potential specialist applications over strain EVE, which appears more generalist. This highlights the importance of strain choice prior to establishing a selection programme. Interestingly, Industry-strain-A was previously identified as harbouring signatures of admixture, suggesting that the observed genetic diversity may also include contributions from hybridisation between genetically distinct parental lineages (Generalovic et al., 2023). Hybridisation could also be used in breeding, both to increase baseline genetic diversity and potential for yield increases. It is notable, for example, that heterosis in black soldier fly populations has been shown to increase larval sizes by up to 32% (Meyermans et al., 2025).

Body size heritability highlights genetic potential for selective breeding in the black soldier fly

We present the first evidence for a quantitative trait of economic and evolutionary importance having a heritable genetic component within the black soldier fly. Larval (h^2 95% CI: 0.58–0.77) and prepupal body size (h^2 95% CI: 0.72–0.84) were both highly heritable. It is notable that Hull et al. (2024) obtained a much lower estimated heritability of larval weight phenotypes (0.18); however, these data appeared to be heavily affected by genetic drift, and Single-nucleotide polymorphism (SNP)-based methods can also substantially underestimate values (Lee et al., 2015). This, combined with the fact that long-term artificial selection has been successfully performed on black soldier fly (Facchini et al., 2022), does suggest that there is genetic variation within strains that can be used for improvement through selective breeding.

Life-history traits show potential diet-specific trade-offs in the black soldier fly

Our data suggest that interactions between quantitative traits, such as harvest weights and development, exist across strains indicating potential pleiotropy (Reshma & Das, 2020). Complex quantitative traits often show many epistatic effects (Mackay, 2014). For example, eight genomic regions across two chromosomes interacted to influence thorax length in *D. melanogaster* (Calboli et al., 2003). The identification of quantitative trait loci (QTL) is the first step towards identifying the genes underlying complex phenotypes with agronomic importance (Dekkers & Hospital, 2002). Highly correlated traits may also be used to inform selective regimes; for example, larger harvest weight selection may lead to slower development but also greater waste reduction ability. Therefore, trade-off effects using specific diets must be assessed prior to establishing selection programmes. Despite an absence of evidence for trade-offs for increased larval size in published artificially selected black soldier fly populations (Facchini et al., 2022), interacting traits, such as development will require further genomic and phenotypic investigation prior to widespread deployment of these programmes at this early stage in the industry.

CONCLUSION

This study explored the genetic and phenotypic variability of several quantitative traits of evolutionary and economic importance in the black soldier fly, providing insights and opportunity to leverage this variation for selective breeding. We detected significant genotype-by-environment effects demonstrating that environmental factors, such as diet significantly influence phenotypic outcomes depending on genetic background. These G × E interactions are also of considerable academic and industrial interest, with our data supporting previous findings. Therefore, the black soldier fly provides an interesting system in which to study the roles of plasticity and adaptation in generating phenotypic variation. Whilst supporting previous evidence of G × E effects, we show for the first time the considerable variation within strains. Variation within and between families suggests that both strain and family-based selection methods may be successful. Characterising phenotypic diversity within this system also suggests strain-specific adaptation to organic waste that could be used to enhance commercial performance. We also identified the first estimate showing high heritability for body size, indicating a foundation for future breeding programmes. Finally, we also identified the first evidence for a diet-specific trade-off effect between harvest weights and development, raising concerns over off-target effects during selection. Overall, these results suggest that the current practice of a one-strain-fits-all approach is not the optimal solution for the industry and may be detrimental in some contexts. This study provides a foundation for harnessing variation and identifying heritable traits of economic importance that will enable the insect livestock industry to progress selective breeding to improve productivity.

AUTHOR CONTRIBUTIONS

Tomas N. Generalovic: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; validation;

visualization; writing – original draft; writing – review and editing. **Chris D. Jiggins:** Funding acquisition; project administration; supervision; writing – review and editing. **John J. Welch:** Methodology; validation; writing – review and editing. **Sam Leonard:** Formal analysis; investigation. **Christoph Sandrock:** Formal analysis; writing – review and editing. **Miha Pipan:** Funding acquisition; supervision. **Tarryn Schuldiner-Harpaz:** Formal analysis; validation.

ACKNOWLEDGEMENTS

T.N.G. was supported by the Biotechnology and Biological Sciences Research Council (BB/M011194/1). This study was supported in kind by Better Origin. We also thank Dr. Erika De Castro and Dr. Luca Livraghi for supporting the final data collection phase.

CONFLICT OF INTEREST STATEMENT

Miha Pipan is the Chief Scientific Officer of Better Origin.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo https://doi.org/10.5281/zenodo.14889739 (Generalovic et al., 2025) reference number: 14889740.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Linear model correlation between body size (cm^2) and weight (g) of black soldier fly (*Hermetia illucens*) pupae used to verify image analysis method to estimate body size of larval and prepupal stages. Shaded area represents the 95% confidence interval. Result shows a significant and strong relationship between area (a proxy for body size) of an individual and the weight validating the method of phenotyping.

Table S1. Conditions for experimental procedures performed using black soldier fly (*Hermetia illucens*) populations in this study.

Table S2. Full statistical model output from black soldier fly (*Hermetia illucens*) life-history trait linear mixed effect and generalised linear mixed models supporting Table 1.

Table S3. Effects of family-derived variation impacting body size of black soldier fly (*Hermetia illucens*) strains.

How to cite this article: Generalovic TN, Sandrock C, Leonard S, Schuldiner-Harpaz T, Pipan M et al. (2025) Variation in strain performance and estimates of heritability of body size indicate considerable potential for genetic improvement of the black soldier fly (*Hermetia illucens*). *Entomologia Experimentalis et Applicata 00*: 1–17. https://doi.org/10.1111/eea.13565