# 613. Genetic parameters of black soldier flies estimated in full sib design

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# Abstract

The aim of this study was to design a scheme for black soldier flies (BSF, *Hermetia illuscens*) to collect data and estimate genetic parameters for the recorded traits. A full sib family design was implemented in a practically feasible way to record multiple traits on the same individuals across a number of families. Genetic parameters were estimated using a linear mixed model. Heritability estimates for body weight at prepupa, body weight at pupa, development time to prepupa was 0.25, 0.45 and 0.38, respectively. The common environmental effect was large at the larval stage and reduced after housing the BSF individually. In general, body weight showed a positive phenotypic correlation between the S2, prepupa and pupa stages, and development time was positively correlated with body weight at (pre)pupa stage. In conclusion, a full sib family design is a practically feasible way to collect data for estimation of genetic parameters in the BSF.

# Introduction

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Insects are an alternative protein source that can add to the challenge in the food transition we are facing today. Insects can be used as feed for pigs, chickens and fish, or directly for human consumption. In both cases, large quantities of the tiny bugs are needed to obtain sufficient product. Phenotypic selection of larger individuals is effective in insect systems to increase mass (Morales-Ramos *et al.*, 2018). Phenotypic selection for morphological traits is in general very effective, as body mass has a moderate to high heritability in most species (e.g. Visscher *et al.*, 2008). However, for most insect populations produced for edible proteins the genetic parameters are unknown, and neither are their unfavourable genetic correlations with other important traits. Insect species have the major limitation that they are difficult to track individually over time, which makes it difficult to collect suitable datasets similar to what is common in larger livestock species. Black Soldier Flies (BSF, *Hermetia illuscens*) can upcycle low-quality organic waste-streams into high-quality proteins and are currently the most used species for livestock feed. Therefore, the aim of this study was to design a scheme for BSF to collect data and estimate genetic parameters for the recorded traits.

# Materials & methods

**Data collection design.** A full sib (FS) family design was implemented in a practically feasible way to record multiple traits on the same individuals across a number of FS families (Figure 1). Data was collected in 5 batches at a commercial BSF facility. At random, 5 to 10 fresh egg clutches were collected in each batch from the general population, assuming each clutch is one FS family. Each clutch was split in half and each half was placed in a container with standard feed. Hence each FS family was housed in two groups (i.e. two common environments) under similar conditions. Please note that the common environment is nested within family, as mixing of families was impossible due to lack of identification. After 10 days, 30 larvae were individually weighted from each family-environment combination (Body Weight Stage 1, BW\_S1), and housed individually in tubes with standard feed. They were individually weighted again after 7 days (Body Weight Stage 2, BW\_S2). At that moment, half of the 30 individuals per family-environment combination were freeze dried, and weighted afterwards (Dry Weight Stage 2, DW\_S2), they were stored for



Figure 1. Schematic overview of the data collection.

other purposes. The remaining 15 individuals per family-environment combination were kept individually for further measurements. Every two days they were checked to see if they had reached the prepupa stage (black but still mobile), and if so they were weighted (Body Weight prepupa, BW\_prepupa), and the day was recorded. The number of days from start of the batch till weight measurement of prepupa was included as a trait to represent development time to prepupa (Development Time, DevTime). The final pupal weight (black and immobile) was measured 12 days after reaching prepupa stage (Body Weight pupa, BW\_pupa). Furthermore, it was recorded if they emerged to adults, and what their sex was based on adult morphology.

**Estimation of genetic parameters.** Genetic parameters were estimated using the following linear mixed model in ASReml (Gilmour *et al.*, 2015):

$$y = Xb + Zfam + Wc + e \tag{1}$$

Where *y* is the vector of observations, *b* vector of fixed effects, including the mean, sex as a fixed regression, with males being 0, females being 1, and individuals with unknow sex (the ones freeze dried and nonemerging) were included as 0.5 assuming a 50/50 sex ratio. Batch was included as a random effect. BW\_S1 was not measured in batch 1. For BW\_prepupa a fixed regression was included on number of days till prepupa weight measurement (i.e. DevTime). *fam* is the vector of random family effects, *c* is the vector of common environment nested within family, and *e* is the vector of random residual effects. The *X*, *Z* and *W* are the design matrices relating observations to the respective levels of fixed and random effects. Random effects were assumed to be normally distributed:  $fam \sim N(0, I\sigma_{fam}^2)$ ;  $c \sim N(0, I\sigma_c^2)$ ;  $e \sim N(0, I\sigma_e^2)$  with I as identity matrix.

The additive genetic variance  $(\sigma_a^2)$  is estimated as two times the family variance  $\sigma_{fam}^2$ . The heritability was estimated as:

$$h^2 = (2 * \sigma_{fam}^2) / (\sigma_{fam}^2 + \sigma_c^2 + \sigma_e^2)$$
<sup>(2)</sup>

The contribution of the common environment to the phenotypic variation was estimated by:

$$c^{2} = (\sigma_{c}^{2})/(\sigma_{fam}^{2} + \sigma_{c}^{2} + \sigma_{e}^{2})$$
(3)

Phenotypic correlations were estimated in a bivariate fashion with model 1 excluding the family and common environmental components. The dataset was too small to estimate genetic correlations.

#### Results

In total 2,731 larvae were recorded, of which 1,285 were followed until emergence to adulthood. A total of 6 traits were measured, their mean is given in Table 1.

Heritability estimates are given in Table 2, the three weight traits at larval stage had large standard errors, hence we were not able to declare them significantly different from zero. From Table 2 it can be seen that the common environmental effect was largest on the first body weight measure (BW\_S1) and reduced substantially when individuals were housed individually and as time between group housing and trait measure increased.

The strongest phenotypic correlation (0.81) was found between prepupa and pupa body weight (Table 3), which were both unfavourably correlated with DevTime.

Trait	Ν	Fam <sup>1</sup>	Mean (sd)	Min	Max
BW_S1 (mg)	2,318	39	6.62 (4.32)	0.25	39.01
BW_S2 (g)	2,731	44	0.23 (0.05)	0.01	0.35
DW_S2 (g)	1,431	44	0.07 (0.02)	0	0.12
BW_prepupa (g)	1,285	44	0.22 (0.04)	0.08	0.34
BW_pupa (g)	1,288	44	0.17 (0.03)	0.02	0.25
DevTime (d)	1,285	44	22.51 (1.25)	21	27
<sup>1</sup> Number of families.					

Table 1. Descriptive statistics of measured traits in commercial BSF.

#### Table 2. Heritability (h<sup>2</sup>) and common environmental effect (c<sup>2</sup>) of the traits, with their standard error in brackets.

Trait	h2	<b>c</b> <sup>2</sup>
BW_S1	0.35 (0.72)	0.71 (0.13)
BW_S2	0.32 (0.25)	0.43 (0.08)
DW_S2	0.24 (0.27)	0.44 (0.09)
BW_prepupa	0.25 (0.11)	0.17 (0.04)
BW_pupa	0.45 (0.12)	0.14 (0.04)
DevTime	0.38 (0.16)	0.25 (0.06)

Trait	BW_S2	BW_prepupa	BW_pupa	DevTime
BW_S1	0.33 (0.02)	-0.007 (0.03)	-0.01 (0.03)	-0.38 (0.03)
BW_S2		0.39 (0.02)	0.49 (0.02)	-0.23 (0.03)
BW_prepupa			0.81 (0.01)	0.62 (0.02)
BW_pupa				0.41 (0.02)

### Discussion

Insects are very small and hard to identify individually over time, which is the main limitation for the estimation of genetic parameters, and even more so for genetic selection programs. The FS family design presented here is practically feasible, as it allows to collect data on individuals from different families not knowing the parents. Ideally multiple families were housed in the same group to disentangle the common environmental effect further. A maternal effect may be present in insects as the conditions under which the mother developed and laid her eggs potentially play a role (Mousseau and Dingle 1991), but the applied design did not allow to estimate maternal effects.

For a general full sib design we estimated that with 15 individuals from 45 families we would be able to estimate heritabilities of 0.3 with a standard error around 0.08 (following Falconer and Mackay, 1996). The obtained standard errors of our experiment were higher, as there are always factors that cannot be controlled in real life. However, reaching standard errors of 0.11-0.16 for the traits measured later in the lifecycle was satisfying. Collecting data on more families will improve the heritability estimates further.

The standard errors of the heritability were higher for the traits at larval stage. At the larval stages the common environment played an important role in the phenotypic variation observed. The common environmental factor is only a short common environment from egg clutch collection till 10 days thereafter, closest to the larval stage, which explains the large common environmental effect at the larval stage. The common environmental impact reduced as the individuals were housed individually for longer time. However, at the later life stages the differences in individual environment may be confounded with individual genetic potential, even though it was averaged within family. Taken this together with the fact that the family variance also includes a potential maternal effect, the heritability estimates are likely somewhat overestimated.

The positive phenotypic correlation between body weight at S2, prepupa and pupa stages can be exploited to select for body weight at an earlier developmental stage. The positive correlation between body weight at (pre)pupa and development time is unfavourable, as this would increase the generation interval. Considering profitable protein production, body weight and development time should be weighted in a selection index to obtain an optimum in the total mass produced per year for instance.

In conclusion, a FS family design is a practically feasible way to collect data for estimation of genetic parameters. The number of families measured is an important factor in decreasing the standard error of the estimates, in practise more families are needed than estimated theoretically, due to uncontrollable factors that play a role in practical situations.

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