Towards selective breeding in insects – estimating genetic parameters with individual-level phenotypes and pedigree

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Abstract
The rapid life cycle of most insects allows for the direct comparison of simulated and realised genetic gain from insect breeding schemes, enabling real-time optimisation of the breeding scheme design. However, rearing and maintenance of insects gives rise to major challenges due to specific dietary and environmental requirements, complex life cycles and reproductive patterns and this adds complexity to all aspects of the breeding plan implementation. We have developed an experimental design for large-scale individual phenotyping and tracing pedigree records and applied this to estimate genetic parameters for larval size and egg-to-adult development time using the housefly (*Musca domestica*) as a model species. These parameters contribute valuable information on the potentials for genetic improvement in this species and the methods and results presented can be used more generally in the process of insect breeding scheme implementation and optimisation.

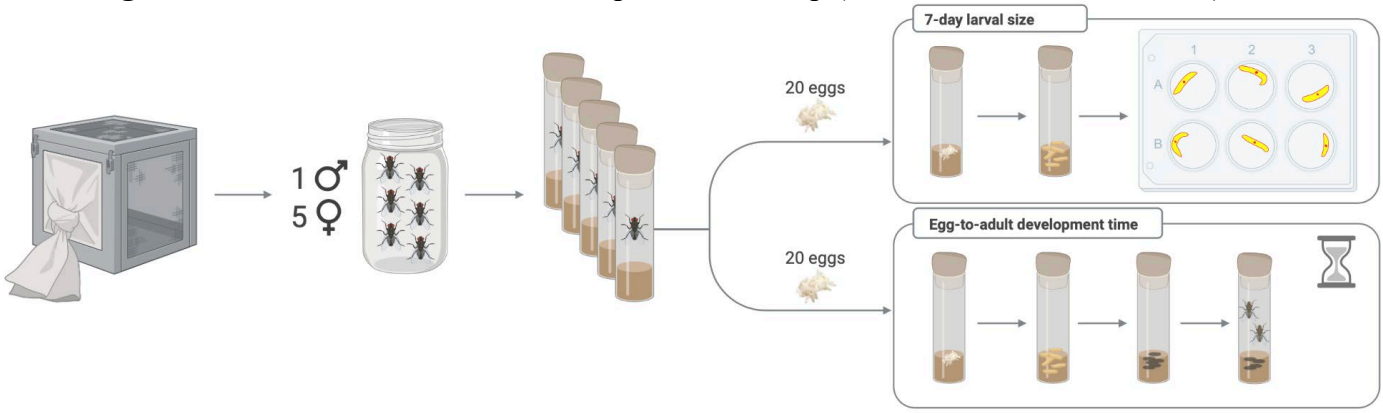
Introduction
Using insects as food and feed has received renewed attention in the last decade, partly due to the ability of many insect species to utilise low-value waste products and convert this input into high-value protein for a more sustainable food and feed production (see van Huis and Oonincx, 2017, for a review). This has initiated an interest in the optimisation of insect farming through the manipulation of environmental factors (Tomberlin and Cammack, 2017). Selective breeding has historically been an important tool in the improvement of crop and livestock production but has so far received little attention within insect production (Eriksson and Picard, 2021). Many insect species are short-lived and highly fecund, theoretically enabling rapid and transgenerational optimisation of production efficiency through selective breeding. Variance components and heritabilities of economically important traits are key indicators of the potential for successful genetic improvement (Hill, 2010). Insects pose a challenge when attempting to estimate such parameters partly due to their rearing requirements, fragility, mode of reproduction and, occasionally, metamorphic life cycle. These fundamental challenges call for novel methods and protocols which can be applied in insect breeding schemes. In this study, we construct a two-generation pedigree by implementing a full-sib/half-sib mating design using the housefly (*Musca domestica* L.) as a model organism for holometabolous dipterans, an insect order highly suitable for converting organic waste into valuable protein products (Pastor et al., 2015). We then estimate genetic parameters for two production-relevant traits, larval size (LS) and egg-to-adult development time (DT), using a large-scale laboratory experimental setup, designed to fit the unique biology and life cycle of holometabolous insects.

Materials and methods
Population. The housefly population used in the experiment was an outbred population established in June 2021 with flies collected from six cattle farms distributed across Denmark.

The population was since maintained in the lab at a census size of ~15000 individuals divided into three replicate populations of ~5000 individuals, maintained in fly cages (30x30x30 cm) with ~1000 flies in each cage. In every generation, offspring from the five cages of a replicate population were intermixed at the pupal stage and randomly distributed into new cages. Flies were reared under standard laboratory conditions at 23°C, 40-60 % relative humidity with a photoperiod of 12:12 h (light:dark) throughout their lifecycle. Larvae were reared on a standard laboratory larval medium composed of wheat bran, alfalfa meal, dry yeast, malt and tap water and adults had access to water and a petri dish filled with equal parts of granulated sugar, icing sugar and powdered milk.

Family generation. A nested paternal half-sib/full-sib design was used when generating the families in this experiment (**Figure 1**). The parent-generation was established by anesthetising and sorting newly emerged flies every 24 hours into sex-, age- and replicate-specific virgin cages. Virgin flies had access to granulated sugar, icing sugar, powdered milk and water.

Figure 1 Schematic illustration of the experimental setup (created with BioRender.com).



At the age of 10 d ± 12 h, one male and five female flies were sorted into glass jars (H: 11.5 cm, Ø: 6 cm) for mating. Each jar was sealed with a foam stopper fitted with a micro centrifuge tube with 10% sugar solution. From the three replicate populations, 30, 30 and 19 mating jars were established over several days depending on when the flies reached the age of 10 days. A large number of virgin flies from replicate 3 were lost due to a technical error. Therefore, a second round of matings from replicates 1 and 2 were established with 14-day old virgin females to compensate for the reduction in family number from replicate 3. A total of 94 mating jars were established when combining all replicate populations. Flies were allowed to mate for 30 hours, where after all flies in each jar were anesthetised and females were sorted into oviposition vials (H: 9 cm, Ø: 2.5 cm) with 3 g fresh medium and kept in climate chambers at 23°C, 60-70 % relative humidity under a 12:12 h (light:dark) photoperiod. Oviposition vials were subsequently checked for eggs every 12 hours until the female had either oviposited >20 eggs in one oviposition event or died. If <20 eggs were found, females were transferred to a new oviposition vial with fresh medium. If >20 eggs were found, these were distributed into new vials with 10 g of fresh medium. The first 20 eggs were designated for the measurement of LS, and the next 20 eggs were designated for the measurement of DT, ensuring that the two traits were recorded on full siblings. Eggs for the two traits were always collected from the same oviposition event and if a female only oviposited enough eggs for one group, then no eggs were collected to measure DT. The offspring were subsequently reared under the same conditions as the females. A total of 256 families were established to measure LS (94, 107 and 55 for replicate 1, 2 and 3, respectively), and 244 families were established to measure DT (87, 103 and 54 for replicate 1, 2 and 3, respectively).

Phenotyping. LS was measured at day seven of the offspring life cycle using an automated size estimation procedure as described in Laursen et al. (2021). Full siblings from a vial were distributed individually into a numbered well-plate and mean larval surface area for all larvae in each family was acquired using a 60 s acquisition time. The numbering of the plate allowed individual ID-assignment. DT was estimated every 12 hours by checking vials for newly emerged adults, which were removed from the vials and sexed. Individual IDs were assigned, and development time calculated for each emerged adult (± 12 h). All vials were continuously checked until four days had passed where no flies emerged.

Statistical analysis. Variance components for LS was estimated with a univariate linear Gaussian sire-model using the full-sib family mean:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{s} + \mathbf{e} \quad (1)$$

and DT was analysed using a univariate linear Gaussian animal-model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{vial} + \mathbf{e} \quad (2)$$

where **y** was the vector of observations of either mean LS or DT; $\boldsymbol{\beta}$ was a vector of fixed effects including replicate, ID of the person sorting the eggs (four groups), sex (only for DT) and dam age (2 groups, only for LS); **s** was the vector of random sire genetic effects, $N(0, \boldsymbol{I}\sigma_s^2)$; **a** was the vector of random animal additive genetic effects, $N(0, \boldsymbol{A}\sigma_a^2)$; **vial** was a vector of random rearing vial effects, $N(0, \boldsymbol{I}\sigma_{vial}^2)$; **e** was the vector of random residuals, $N(0, \boldsymbol{I}\sigma_e^2)$; $\boldsymbol{I}$ was the identity matrix, $\boldsymbol{A}$ was the additive genetic relationship matrix, and **X**, **Z**, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ were the corresponding incidence matrices. Narrow-sense heritabilities were estimated as $h^2 = \frac{4\sigma_s^2}{\sigma_s^2+\sigma_e^2}$ when using the sire model, and as $h^2 = \frac{\sigma_a^2}{\sigma_a^2+\sigma_{vial}^2+\sigma_e^2}$ when using the animal model. Variance components were estimated using REML with the average information algorithm (AI-REML) implemented in the DMU software package (Madsen et al., 2006; Madsen and Jensen, 2013).

Results

LS was measured on 1,725 larvae from 184 dams representing 73 sires, and DT was estimated for 630 flies from 97 dams representing 21 sires. The number of larvae measured in each full-sib family ranged from 1-20 larvae, and the number of adult offspring emerging ranged from 1-17 flies. Descriptive statistics for both traits are presented in **Table 1.**

Table 1 Descriptive statistics for the traits mean 7-day larval size (based on full-sib family means) and egg-to-adult development time in houseflies (*Musca domestica*).

Trait	Unit	No. records	Mean	SD	Median	Min	Max
Mean 7-day larval size	mm^2	184	15.99	8.19	16.24	2.63	30.81
Egg-to-adult development time	Hours	630	500.29	45.17	494.0	422.0	686.0

The estimated heritabilities (**Table 2**) were moderate for both LS and DT. The random effect of rearing vial contributes with a large portion of the total variance for DT.

Table 2 Estimated genetic (σ_a^2), rearing vial (σ_{vial}^2) and error (σ_e^2) variances and heritabilities (h^2) for mean 7-day larval size and egg-to-adult development time with the corresponding standard error SE(h^2) from univariate trait analysis.

Trait	$\boldsymbol{\sigma_a^2}$	$\boldsymbol{\sigma_{vial}^2}$	$\boldsymbol{\sigma_e^2}$	$\mathbf{h^2 \pm SE(h^2)}$
Mean 7-day larval size	6.05	-	46.70	0.13 ± 0.33
Egg-to-adult development time	480.42	1,019.92	453.00	0.25 ± 0.44

Discussion

This study aimed at estimating variance components and heritabilities for two production relevant traits in housefly larvae and adults, using a complex mating design frequently applied in aquacultural species (Gjerde et al., 1996). Few studies have presented genetic parameters for traits relevant for protein production in holometabolous species such as the housefly (e.g. Bryant and Meffert, 1998). Estimating genetic parameters for holometabolous species is challenging, partly because physical tagging for individual identification is impossible due to metamorphosis, optimal rearing conditions and environmental susceptibility are life-stage specific and isolated rearing introduces undesired variation, all of which are required to obtain accurate phenotypes from individuals with known genetic relationships. We present moderate heritability estimates for the two investigated traits, but the challenges are reflected by the large variation around the estimates. Additional challenges will arise in coupling phenotypes from different life-stages to estimate genetic correlations. We have approached this issue by designing the experiment so that the two traits were obtained from full-sibs from the same oviposition event. The methods and results presented here constitute the first steps towards the practical implementation of insect breeding plans. Next steps include estimation of the genetic correlations between DT and LS and other traits relevant for insect production (work in progress). Subsequently we will evaluate the potential of genetic gain of selective breeding through simulation studies and compare this with the realised gain obtained from laboratory selective breeding.

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