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RESEARCH ARTICLE

Exploring the potential of crossbreeding to enhance black soldier fly (*Hermetia illucens*) production

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Abstract

The increasing world population leads to a growing demand for animal protein, calling for sustainable solutions. Insects, such as the black soldier fly (*Hermetia illucens*, BSF), will therefore play a crucial role due to their fast growth, high protein content, good bioconversion efficiency and their ability to thrive on diverse feed substrates. Selective breeding has proven valuable in plants and livestock and holds great potential for farmed insects. Moreover, crossbreeding is a successful strategy to improve the genetic value of progeny, for example by exploiting heterosis. Nevertheless, crossbreeding experiments in BSF remain scarce. In this exploratory study, we present the outcome of a crossbreeding experiment in BSF. Four strains (F0) were used to set up reciprocal crosses (F1). Subsequently, four second generation populations (F2) were created. All populations were reared at pilot scale on chicken feed in triplicate. Each generation (F0, F1, and F2) was evaluated for growth, feed conversion, bioconversion efficiency, mating behaviour and egg production. The mean final larval mass of the F2 generations did not significantly differ from the mean of the F0s, although the development speed of the larvae was higher, resulting in a higher percentage of prepupae at the end of the rearing phase. Interestingly, some F1 crosses showed reciprocal differences (i.e. reversing maternal and paternal lines) in maximal larval weight of 32%. Furthermore, egg production was 19% higher in the F2 generations compared to the average F0s. Taking both production and reproduction traits into account, we calculated an increase of 16.5% in total larval mass production and an increase of 19.1% dried larval mass production for the F2s compared to the F0s. These findings explore the potential for crossbreeding in future BSF rearing programmes and highlight the need for further investigation into reciprocal differences between parental lines.

Keywords

development speed – genetics – heterosis – reciprocal differences – selection

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1 Introduction

Insects have emerged as an alternative resource for animal feed and various valuable by-products (Barragan-Fonseca *et al.*, 2017). Moreover, they offer a promising solution for organic waste recycling and bioremediation (Čičková *et al.*, 2015; Lalander *et al.*, 2015). Therefore, insect farming is rapidly establishing itself as a new agricultural subsector, particularly for feed applications in aquaculture and monogastric livestock production (Makkar *et al.*, 2014; Gasco *et al.*, 2019; Facchini *et al.*, 2022). Conventional animal feed ingredients, especially protein sources, often have a significant ecological footprint. The sustainability of animal feed could be substantially increased by using insect-based components derived from recycled nutrients. Among farmed insects, the black soldier fly (BSF), *Hermetia illucens*, stands out as the most widely cultivated species globally (Tomberlin and van Huis, 2020). BSF larvae-based products are particularly valued as feed ingredients for pigs (DiGiacomo and Leury, 2019; Veldkamp and Vernooij, 2021), poultry (Dorper *et al.*, 2021), and fish (Henry *et al.*, 2015). BSF larvae have a remarkable bioconversion efficiency, fast growth, high protein content and ability to thrive on a diverse range of feed substrates (Belperio *et al.*, 2024; Jin *et al.*, 2022; Siddiqui *et al.*, 2024). As the industry grows, optimizing productivity becomes increasingly important.

Genetic optimization through selective breeding is a common practice in crops, livestock and aquaculture, and is currently gaining attention in farmed insects. For BSF, Facchini *et al.* (2022) demonstrated that long-term genetic mass selection for (up to) 16 generations can substantially increase larval weight (+39%), dry matter (+26%), and protein content (+32%). Hull *et al.* (2024) estimated heritabilities based on single nucleotide polymorphism (SNP) marker information for larval mass at 0.18, for clutch size at 0.06, for hatchability at 0.16. Both studies suggest significant potential for genetic improvement of BSF larvae. Also in house fly (*Musca domestica*), Hansen *et al.* (2024) estimated low to intermediate heritabilities for larval size (0.10) and development time (0.25). Given these relatively low heritabilities, improvement might be most efficient via crossbreeding. In animal and plant breeding, crossbreeding has been widely employed. It involves mating individuals from genetically different populations with the aim to increase the overall performance of offspring. Crossbreds often benefit from heterosis (hybrid vigour) which indicates that the (crossbred) offspring outperform the average of the parental populations. Heterosis is particularly present for traits with relatively low heritabilities, such as reproductive and developmental traits, and is expected to result from non-additive genetic effects (Falconer and Mackay, 1996). By combining the strengths of distinct genetic lines, crossbreeding can produce hybrids with improved characteristics such as increased resilience to environmental stresses, enhanced growth rates, and better overall (re)productivity (Lippman and Zamir, 2007). However, the choice of the parental lines and the setup of the crossbreeding scheme is important. For example, crossbreeding in commercial pig production maximizes growth performance in paternal lines and maternal capabilities and litter size in maternal lines (Nelson and Robison, 1976). Similarly in insects, crossbreeding in *Tenebrio molitor* mealworms showed differences in egg production and hatch rates for different crosses (Adamaki-Sotiraki *et al.*, 2023). However, the potential of crossbreeding in BSF is currently not reported.

Various studies have already explored the existence of different BSF populations genomically. Kaya *et al.* (2021) identified 16 genetic clusters within the global BSF population, each composed of different populations with substantial genetic differences. Ståhls *et al.* (2020) and Guilliet *et al.* (2022) used mitochondrial genomes to explore BSF diversity. It was found that North American strains are the ancestors of most current commercial BSF strains (Kaya *et al.*, 2021). Moreover, Rhode *et al.* (2020) showed that BSF populations with a small effective population size are prone to inbreeding depression, which can lead to population crashes.

In this exploratory study we conducted a crossbreeding experiment with four different BSF strains, including reciprocal crosses to assess the potential of crossbreeding as a way of genetic improvement. Exploring crossbreeding in insect breeding programmes holds significant promise for advancing the agricultural sector and maximizing the benefits of insect-derived products.

2 Material and methods

BSF rearing

BSF rearing was conducted at the Insect Pilot Plant (Thomas More University of Applied Sciences, Kleinhoefstraat 4, Geel, Belgium) between September and December 2023. There, facilities are in place to rear and process insects at both laboratory and pilot level (Frooninckx *et al.*, 2023). Before the start of the experiment, all BSF populations were continuously propagated at the research facility for at least 15 generations

on chicken start mash under uniform rearing conditions and were therefore considered stable (Broeckx *et al.*, 2025). A schematic overview of the experimental setup and rearing process is given in Supplementary Figure S1.

Each rearing started with freshly harvested eggs (1 g ± 0.011 g; ±36,000 eggs (Booth and Sheppard, 1984)), all deposited in a time span of 48 h. The eggs were placed in a plastic container (D 11.5 × H 13.7 cm) on 100 g chicken start mash (Chicken Start Mash 259, AVEVE, Belgium) mixed with 100 g water (total dry matter content of ±45%). The container was incubated in a climate chamber at 27 °C and 60% RH. After four days, the eggs hatched and the larvae and frass were transferred to a larger container with 240 g of chicken start mash and 360 g of water. Nine days after egg harvest, the larval density was measured by counting the number of larvae in five subsamples of 1 g (larvae + frass) homogenized in a 6-L drum bucket spinning at 40 RPM. This larval density was used to distribute ± 7,500 larvae to each rearing container (W 40 × L 60 × H 30 cm) which contained 7.3 kg feed (3.3 kg chicken start mash and 4 kg of water). This moment was considered the start of the rearing phase and was called day 0. BSF larvae were monitored daily until day 10. At each day, individual larval weight was measured by randomly subsampling and weighing 25 larvae from each replicate. On day 10, larvae were separated from the frass and each fraction was weighed. A random subsample of 50 larvae was weighed individually, and their development status was checked (prepupae/larvae ratio). The total amount of larvae at harvest was calculated based on the average larval mass and subsamples were taken of both the frass and larvae to measure the dry matter content.

Following the rearing phase and harvest on day 10, larvae were reintroduced to their rearing box, and monitored until more than 75% had developed into prepupae. At this stage, water was added, which triggers the prepupae to crawl out of the containers in search of a dry place to pupate. The migrating prepupae dropped into a large collection bin containing multiple layers of kitchen paper, which simulated a dry environment suitable for pupation, allowing for the collection of clean, dry pupae. After approximately 10 days, newly emerged flies were sexed manually at 4 °C. To create a new generation, 500 males were placed with 500 females in tents (60 × 60 × 90 cm tents, environment 27 °C – Halogen lights of 400 W, at 23 cm from each tent). First matings were observed two days after the flies were put in the breeding tents (2-4 days after the flies emerged, based on synchronization of the populations). Eggs were laid on stacked wooden plates placed on an odour attractant (100 g chicken start mash + 160 g water). Mating behaviour was assessed for eight consecutive days (five times each day before noon with time intervals of minimum 30 minutes) where each mating was recorded.

For each population and each replicate, bioconversion efficiency (BE), waste reduction (WR) and feed conversion ratio (FCR), following Bosch *et al.* (2020), were calculated at day 10 as:

$$BE = \frac{L_{end,dry} - L_{start,dry}}{D_{dry}} \times 100\%$$

$$WR = \frac{D_{dry} - R_{dry}}{D_{dry}} \times 100\%$$

$$FCR = \frac{D_{dry}}{L_{end,\,fresh} - L_{start,\,fresh}}$$

with $L_{end,\,dry}$ the larval biomass (in grams) at the end after drying, $L_{start,\,dry}$ the larval biomass (in grams) at the start of rearing, D_{dry} the amount of feed (in grams, dry), $L_{end,\,fresh}$ the fresh larval end mass (in grams), $L_{start,\,fresh}$ the fresh larval starting mass (in grams) and R_{dry} the residue (in grams, dry) at harvest. To determine dry weights, samples were placed in an oven at 105 °C overnight. FCR was also computed on day 7 and day 8 to explore the FCR at maximal larval weight. To detect significant differences between different populations and generations, pairwise t-tests ($\alpha = 0.05$) were conducted in R (*t.test*), and where necessary Bonferroni correction was applied to account for multiple testing. All figures were generated using ggplot2 (Gómez-Rubio, 2017).

BSF crossing

The crossbreeding scheme that was used in this study is illustrated in Figure 1. Four distinct parental strains were crossed reciprocally to generate the F1 (Strain 1 & 2 and Strain 3 & 4) and F2 (Strain 1 & 2 & 3 & 4) generations. All crosses were denoted as ♂ × ♀. Strain 1 and 3 originate from a European clade, whereas Strain 2 and 4 originate from an Oceanian clade (Broeckx *et al.*, 2022a,b; Frooninckx *et al.*, 2022). Choice of the different strains was based on previous microsatellite research, which included 15 markers to achieve the most genetically diverse combinations possible (Broeckx, 2025). To prevent pairing of emerging flies before sex separation, all flies were kept in the dark (Tomberlin and Sheppard, 2002). All crosses were repeated in triplicate, except for the F1 Cross 4 × 3 which did not yield sufficient fertilized eggs for triplicate setup, and was therefore set up in duplicate maintaining same larval densities. All generations were reared consecutively.

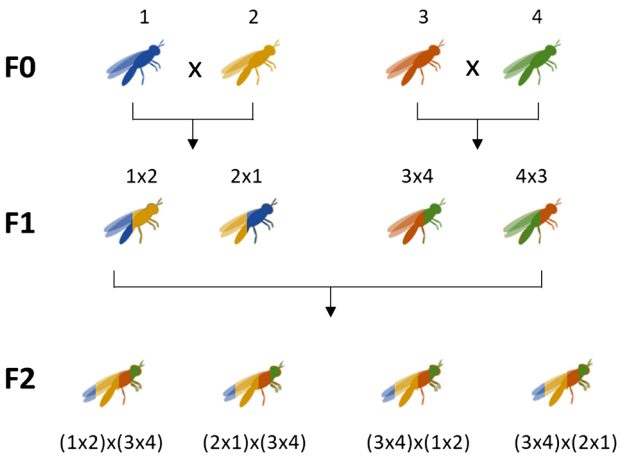

FIGURE 1 Overview of the crossbreeding scheme with four parental strains (F0). Strain 1 and 3 originate from a European clade, whereas Strain 2 and 4 originate from an Oceanian clade. F1 and F2 represent the first and second crossed generation, respectively. All crosses are denoted as ♂ × ♀.

3 Results

Larval production performance

The larval growth curves of all populations are presented in Figure 2. Among the parental lines (F0; Figure 2a), Strain 1 exhibited the highest larval mass (278.12 mg on day 8; SD = 13.06 mg). This growth was significantly larger than in the other parental populations ($P < 0.01$), with Strain 3 showing the lowest maximal larval weight (204.93 mg on day 7; SD = 9.00 mg). The reciprocal F1 crosses (1 × 2 and 2 × 1), did not differ significantly from their parental average. However, in the F1 Cross 4 × 3, an increased maximal larval weight ($P < 0.01$) was observed on day 7 (246.48 mg; SD = 1.70 mg) compared to the parental average (202.05 mg, SD = 7.98 mg) and to the reciprocal Cross 3 × 4 (186.03 mg, SD = 4.71 mg). The F2's showed lower variation, with maximal larval weights ranging between 221.87 mg (SD = 8.68 mg) and 251.28 mg (SD = 16.09 mg) (Figure 2c). Moreover, the F2 generation exhibited accelerated growth, showing a difference from F0 in the growth period from day 5 to day 7 (Figure 2d; Supplementary Figure S4). Total larval production at moment of harvest (day 10) per rearing container is shown in Table 1 and Supplementary Figure S2. Here, no generation-wide significant differences were found between F0, F1 and F2. The larval dry matter content at moment of harvest was significantly higher in generation F2 (36.6%) than in generation F0 (35.8%) ($P = 0.01$).

A summary of the different production parameters for the average of each generation is given in Table 1. The percentage of prepupae at day 10 increased significantly ($P < 0.01$) from F0 (27%, SD = 11%) to F1 (43%, SD = 12%) and F2 (64%, SD = 10%) (Figure 3). FCR on day 7 showed a nearly significant difference between F0 (2.20, SD = 0.31) and F2 (1.94, SD = 0.28) ($P = 0.06$). No significant differences were identified between the FCR of the different populations at maximal larval weight. WR increased significantly ($P = 0.01$) from F0 (63.6%, SD = 5.1%) to F2 (68.2% ± 1.8%) and showed a remarkable reduction in variance. Individual WR per population is shown in Supplementary Figure S3. BE and SR did not differ significantly between the three generations (Table 1).

Reproduction parameters

Observation of the mating behaviour and egg production showed that, on average, male flies emerged sooner than female flies. Oviposition started 4 days after the first hatching of the flies. The cumulative amount of observed matings for each population is shown in Figure 4. For the purebred F0 populations, large differences in number of matings were observed (Strain 1: 200, Strain 2: 43, Strain 3: 122 and Strain 4: 89). For the first crossing (F1), the number of observed matings for both reciprocal crosses of Strains 3 and 4 was higher (182 for 3 × 4 and 160 for 4 × 3) compared to the reciprocal crosses of Strains 1 and 2 (69 for 1 × 2 and 61 for 2 × 1). The cumulative egg production for each population can be found in Figure 5. Although not significant, the average egg production to generate F2 (8.10 g, SD = 1.84 g) was higher compared to the parental strains (F0) (6.77 g, SD = 4.52 g) and the cross to generate F1 (7.07 g, SD = 3.12 g).

4 Discussion

In this research, we explored the effects of crossbreeding in BSF on different production and reproduction parameters. By setting up a reciprocal crossbreeding experiment using four genetic BSF strains from either the European or Oceanian clade, we investigated the potential of BSF crossbreeding. To create the first generation of crossbred flies (F1), we set up reciprocal crosses that showed in some cases reciprocal differences in larval growth. In the second generation crosses (F2), we generated a relatively uniform generation with overall increased reproduction traits and no adverse effects in BE, SR, FCR and WR.

When analysing larval growth performance of the F0s, large differences were observed between the four strains (Figure 2a). This was expected given the diverse

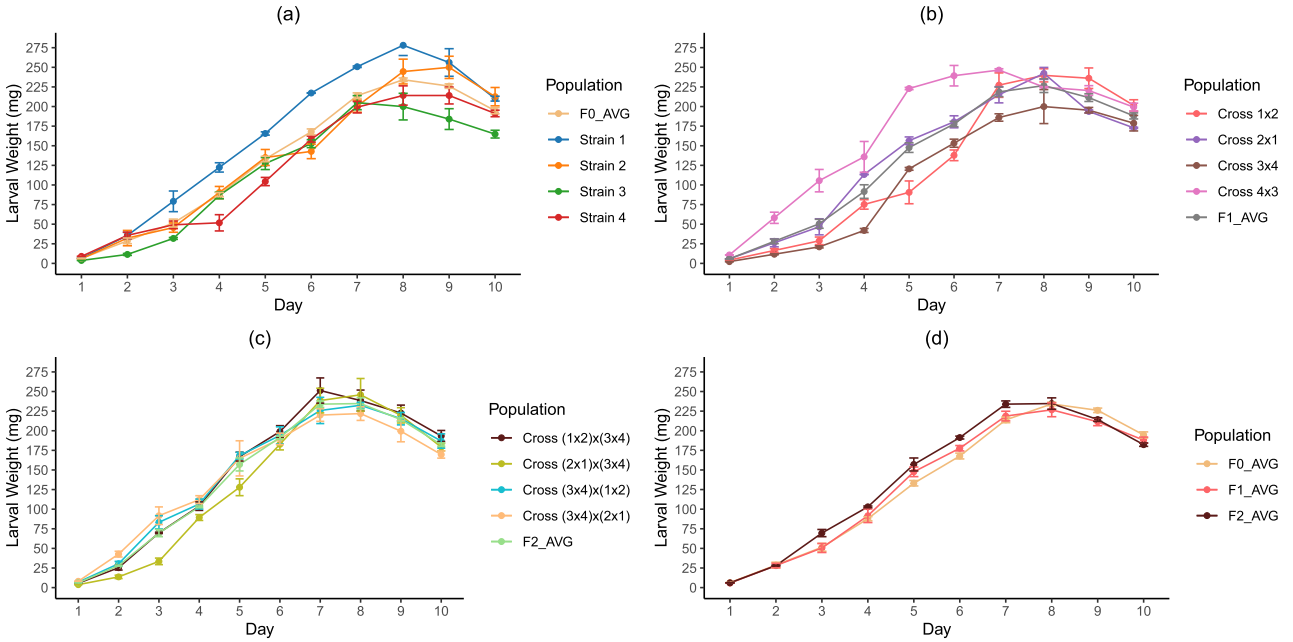

FIGURE 2 Larval growth curves of F0 (a), reciprocal F1 (b) and F2 (c) populations, together with the generation's average (F0_AVG, F1_AVG and F2_AVG, respectively), whereas (d) represents the generation's average larval weight. For each population, the standard deviation of the replicates is indicated with whiskers. Crosses are given as ♂ × ♀.

TABLE 1 Mean and standard deviation (SD, between brackets) of the growth parameters for the average of each generation (F0, F1 and F2)

	F0	F1	F2
Larval weight at day 7 (g, fresh)	213.84 (3.58)	218.71 (6.15)	233.92 (4.11)
Larval weight at day 10 (g, fresh)	194.65 (3.89)	188.07 (3.87)	182.27 (2.15)
Total larval mass per container at day 10 (g, fresh)	1326 (108)	1342 (76)	1272 (107)
Larval dry matter content	0.358 (0.010)	0.365 (0.008)	0.366 (0.004)
Frass dry matter content	0.420 (0.046)	0.471 (0.069)	0.440 (0.030)
Prepupae at day 10 (in %)	27.00 (11.00)	43.00 (12.08)	64.00 (9.80)
FCR at maximal larval weight	1.97 (0.23)	1.95 (0.15)	1.87 (0.25)
FCR at day 7	2.20 (0.31)	2.11 (0.17)	1.94 (0.28)
FCR at day 10	2.39 (0.19)	2.36 (0.17)	2.49 (0.22)
BE at day 10 (in %)	15.17 (1.17)	15.71 (1.04)	14.92 (1.33)
SR at day 10 (in %)	90.47 (6.11)	93.24 (8.07)	89.53 (10.55)
WR at day 10 (in %)	63.60 (5.09)	64.67 (2.71)	68.23 (1.79)

FCR = feed conversion ratio, BE = bioconversion efficiency, SR = survival rate, WR = waste reduction.

genetic background of the strains and these differences were in line with previous larval growth patterns for the four strains (e.g. Sandrock et al., 2022; Broeckx *et al.*, 2025). This ensured good confidence that used rearing methods are reliable and reproducible. Despite good egg production, the F1 Cross 4 × 3 produced an insufficient number of hatched larvae to set up the experiment in triplicate, possibly due to a mould infection. To obtain similar population densities in the rearing containers, rearing was conducted in duplicate for Cross 4 × 3. Although there is genetic differentiation between both parental strains (Kaya *et al.*, 2021), our microsatellite analyses do not show sufficient differentiation to indicate speciation between the strains (Broeckx *et al.*, 2022a,b, 2025).

For the F1 generation, a clear reciprocal effect was observed for Strain 3 and Strain 4, as presented in Figure 6. There was an difference of 23.27% in terms of

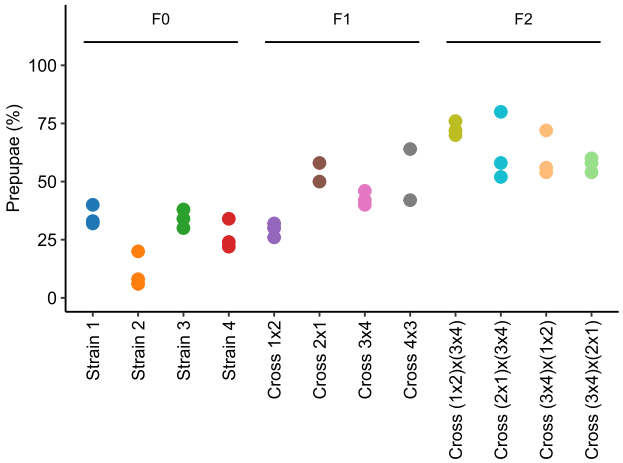

FIGURE 3 Percentage of BSF prepupae present at day 10 in each population.

maximal larval weight for Cross 4 × 3 compared to Cross 3 × 4, which suggests a strong parental (maternal/paternal) effect. This indicates that Strain 4 may possess beneficial maternal traits, which could be further explored for their potential use in BSF breeding programs. If the existence of populations with improved maternal capabilities (e.g. egg production, larval quality, etc.) would be confirmed, this could significantly enhance the efficiency and effectiveness of BSF breeding strategies. Moreover, Cross 4 × 3 outperformed both parental strains (Figure 6a). The average maximal larval weight of the offspring (F1) is higher (235 mg) than that of the parental average (222 mg), which is a clear sign of heterosis. From day 2 on in the rearing process, heterosis is observed for the offspring of Strains 3 and 4 (Figure 6b). Figure 6b clearly shows an increased growth speed and development around day 5 in the offspring generation.

The F2 crosses showed an accelerated growth and faster development compared to the average of the parental populations (Figure 2, significant difference between F2 and F0 on day 5-7). Moreover, the F2 generation had a significantly higher percentage of prepupae at the moment of harvest compared to the average of the parental populations (64% vs 27%; Figure 3). The increased percentage of prepupae explains why the F2 larvae have lost more weight during the last few days, as the larvae enter the prepupal stage after which they dry out and empty their digestive tract (Amrul *et al.*, 2022). An acceleration in development speed, which can be seen as a reproduction trait, as a result of heterosis is not uncommon. This has already been observed in plant species like maize (Hoecker *et al.*, 2006) and insect species like the fall armyworm (*Spodoptera frugiperda*), where the development speed increased by 5.36% after crossbreeding (Lan and Nègre, 2023). Also in livestock, heterosis effects are associated with earlier puberty in cattle (Wiltbank *et al.*, 1966). The observed accelerated larval development in our crossbreeding experiment might be valuable for BSF producers. Such increased speed of development offers the advantage that more rearing cycles can be completed within the same timeframe, thereby increasing the production capacity. Moreover, this could eventually allow for a shortened BSF growth cycle of just 7 days with equal performance, presenting valuable opportunities to better synchronize workloads in rearing facilities. An additional advantage of such shortened growth cycles is the decreased risk of mould infestations on the moist feed substrates. In our experiment, the F2 generation reached maximal larval weight about one day sooner during rearing (–11%). On a lifecycle of 39 days (Supplementary Figure S1), this is a decrease of 2.6%. When comparing the variation in growth performance among the populations in the F0, F1, and F2 generations, it becomes evident that the F2 generation showed less variation between the populations. This could be explained by a more similar genetic makeup present in these four populations, compared to the different F1s and F0 populations. If this uniformity in the F2 could be replicated in follow-up experiments, crossbreeding could be used to create a uniform end-product, which could be beneficial for industrial BSF rearing.

As BSF can play a pivotal role in a more sustainable food and feed system, it is important to evaluate BE for their production (Siddiqui *et al.*, 2022). BE did not differ significantly between the different generations (Table 1). Broeckx *et al.* (2021) measured a BE of 17.6 (SD = 3.4) and a FCR (day 10) of 2.08 (SD = 0.35) on chicken start mash for Strain 1, whereas this strain had an average BE of 16.2 (SD = 1.3) and a FCR (day 10) of 2.19 (SD = 0.13) on chicken start mash in this study. This difference can be explained by the set-up of our rearing system, as we decided to work 'ad libitum' to enlarge the possible difference between the generations. We used 0.4 g dry matter substrate per larvae, whereas Broeckx *et al.* (2021) used 0.2 g dry matter substrate per larvae. It is known that the initial dry matter content plays an important role in both larval growth and waste reduction (Froonickx *et al.*, 2024).

The F2 generation showed a lower variation in matings, compared to the F0 and F1 generations (Figure 4, Supplementary Figure S5). F2 averaged on 128 (SD = 9), whereas F0 averaged at 114 (SD = 66) and F1 at 118 (SD = 62), and F2 showed less variation as evidenced by the lower SD. The crossbred parents from generation

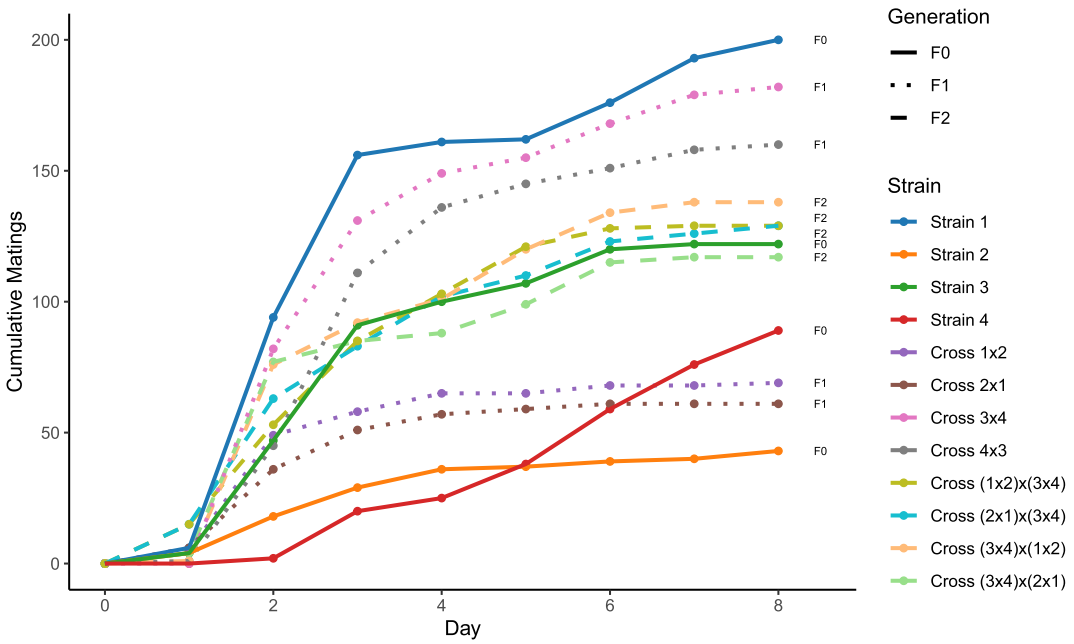

FIGURE 4 Cumulative number of recorded matings for the purebred strains, and the crosses in generations F1 and F2.

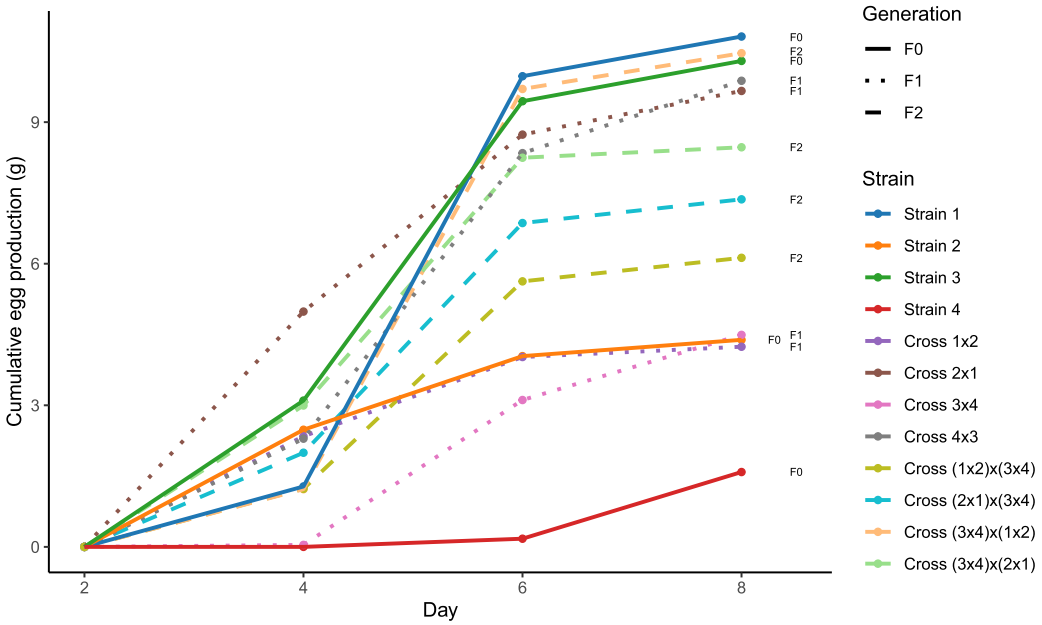

FIGURE 5 Cumulative egg production (in grams) over the 8-day egg laying period for all populations included in the experiment.

F1 showed higher mating abilities with their generation mates, where other purebred populations showed more difficulties. This is a clear exhibit of heterosis where the offspring outperforms the midparent (purebred F0). This study is not the first that detects heterosis on sexual performance, as for example Ekechukwu *et al.* (2015) found that heterosis improved male sexual performance in *Anopheles coluzzii* mosquitos. The majority of the matings in our experiment took place two days after emergence. This might be due to the lack of female acoustic and chemical signals during the first day, as no oocytes are present until two days after emergence (Tomberlin and Sheppard, 2002) and female ovaria are not fully developed yet (Munsch-Masset *et al.*, 2023). Tomberlin and Sheppard (2002) observed maximal oviposition two days earlier, on day 4. This difference might emerge from the density of flies in each mating tent. In our study, the average density was 3,125 flies per m^3, while Tomberlin and Sheppard (2002) used a density of only 115 flies per m^3. According to Tanga *et al.* (2017) and Hoc *et al.* (2019), population density is

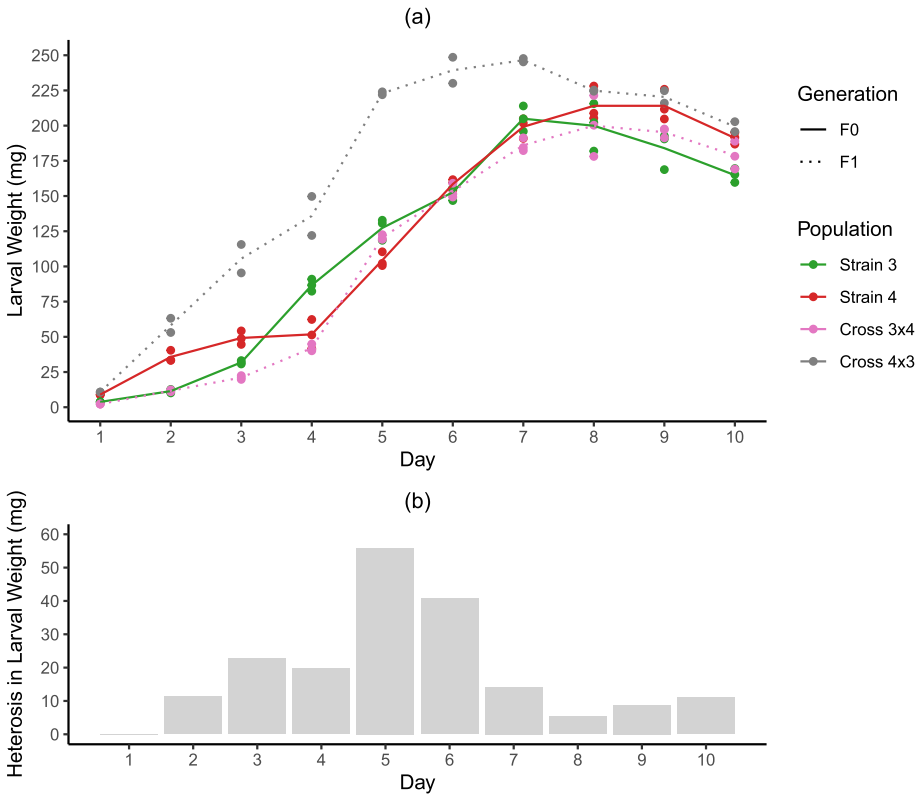

FIGURE 6 (a) Comparison of larval growth curves between Strain 3 and Strain 4, and their reciprocal offspring, Cross 3 × 4 and Cross 4 × 3. (b) Visualisation of heterosis in larval growth per day (in grams), calculated as the difference between the average of the offspring and the average of the parental strains.

an important factor in the mating process, where higher densities result in more matings.

Similarly to the observed matings, the egg production obtained by the crossing of hybrid F1s are much more alike, indicating the strong genetic influence on egg production. An increased egg production by hybrid animals has been observed frequently (Notter *et al.*, 2013), as oviposition in insects is known to be a trait with a low heritability (e.g. in Sand crickets, *Gryllus firmus*; h^2 = 0.20) (Réale and Roff, 2002). The first generation crossing (F1) showed an increased egg production (19.65%) (Figure 5). Heterosis for female fecundity and egg production has already been studied in several insects, like *Drosophila melanogaster* (Ruban *et al.*, 1988) and *Apis mellifera* (Cale and Gowen, 1956), and showed similar patterns.

Table 2 presents the computed total yield for each population, both as total (wet) and dehydrated weight. This computation uses an equal number of parents to start each generation, as done in this experiment, and takes larval survival into account. Cross 4 × 3 showed the highest proportional increase from the parental average. All but one F2 populations outperform the performance of the parental average, with total mass increases reaching up to 171% and dried mass increases peaking at 170%. On average the larval mass of the F2s increased by 16.5% compared to F0, and the total dried mass production increased 19.1% compared to F0. Such increases are important for BSF producers, suggesting that crossbreeding is a promising strategy that warrants further investigation. Such setup of crossbreeding on an industrial scale opens the opportunity for businesses to specially focus on the sale of specific (hybrid) production and reproduction lines to rearing facilities. Although our current setup requires labor-intensive mating and sexing of flies from different populations, this process could be streamlined through automated video monitoring to distinguish between males and females. Additionally, since early-emerging flies predominantly consist of males, and selecting the largest prepupae yields a predominantly female population, the separation of flies based on emerging time could facilitate crossbreeding (Hoc *et al.*, 2019; Broeckx *et al.*, 2025).

The mating design used in this study (Figure 1) enables us to investigate and screen the reciprocal possibilities of BSF crossbreeding while maintaining three replicates for each cross. It should be noted that other mating designs, such as the complete diallel crossing design as for example presented by Yang *et al.* (2024) and Ariyanto *et al.* (2022), use a more complete com-

TABLE 2 Overview of the total mass and larval dry matter content for each container (setup on 1 g eggs), total egg production per population, and the calculated fresh mass and dried mass per population, expressed in absolute values (g) and as difference from the parental average (midparent), for each population and the average of the generation. This calculation considers the larvae survival rate, assumes an average of 36,000 larvae per 1 g eggs (Booth and Sheppard, 1984), and each container was setup with 7,500 larvae (0.21 g eggs)

Generation	Population	Total mass per container (g)	Larval dry matter content	Total egg production (g)	Larval survival rate (in %)	Total mass per population		Total dry mass per population	
						Absolute (in kg)	Difference from midparent	Absolute (in kg)	Difference from midparent
F0	1	1,442	0.352	10.81	91.5	67.9		23.9	
	2	1,351	0.357	4.39	83.8	23.7		8.4	
	3	1,188	0.371	10.30	94.2	54.9		20.4	
	4	1,323	0.352	1.59	92.4	9.2		3.2	
	Average	1,326	0.358	6.77	90.5	38.9		13.9	
F1	1 × 2	1,364	0.362	4.24	88.6	24.4	53%	8.8	55%
	2 × 1	1,264	0.366	9.66	96.4	56.1	122%	20.5	127%
	3 × 4	1,399	0.369	4.49	99.7	29.8	93%	11.0	93%
	4 × 3	1,302	0.362	9.88	87.4	53.5	167%	19.4	164%
	Average	1,332	0.365	7.07	93.0	41.0		14.9	
F2	(1 × 2) × (3 × 4)	1,256	0.366	6.13	100	36.6	135%	13.4	135%
	(2 × 1) × (3 × 4)	1,343	0.365	7.36	86.6	40.8	95%	14.9	94%
	(3 × 4) × (1 × 2)	1,143	0.364	10.46	81.6	46.5	171%	16.9	170%
	(3 × 4) × (2 × 1)	1,346	0.369	8.47	106	57.5	134%	21.2	135%
	Average	1,272	0.366	8.10	93.6	45.4		16.6	

prehensive scheme to disentangle potential heterosis and reciprocal effects. However, such design with four parental populations, would require 16 different crosses (12 reciprocal cross lines and 4 within each population) per replication. Such design, therefore, exceeded the scope of our current exploratory study but may offer valuable insights for future research. However, within our mating design, we were able to explore the potential reciprocal effects between four parental populations. By creating the F2 generation as presented in Figure 1, our goal was to develop a crossbred population that retained roughly 25% of the genetic ancestry from each of the four parental populations. Moreover, when interpreting these results, it is crucial to consider that external factors may have influenced the rearing experiments. Although all rearing actions, measurements, and crossings were conducted in a standardised controlled environment (e.g. temperature, relative humidity, feed), we cannot entirely rule out variations in factors such as seasonal changes, environmental air pressure, and feed composition provided by the supplier. Therefore, repeating these experiments will be crucial to enhance the reliability of our findings. Further investigations could also consider raising the different crossbred generations simultaneously with the four parental strains to better disentangle the possible batch effects. Moreover, in this research, eggs were harvested at day 6 which might influence the length of the generation interval. Collecting eggs earlier (e.g. at day 4) could select females that have quicker oviposition.

Future studies on the potential of crossbreeding in BSF should further investigate the genomic background of the different parental and offspring populations. This could be done by using microsatellites, as described by Kaya *et al.* (2021), or by employing whole-genome sequencing data (Generalovic *et al.*, 2021). Such genotyping could identify the admixture of each parental line in the crossbred offspring. Moreover, such advancements could also allow the screening or prediction of ideal paternal and maternal lines for BSF crossbreeding.

5 Conclusions

In this research, we explored the effects of crossbreeding in BSF. Our findings demonstrate that crossbreed-

ing is feasible in BSF and can enhance production, larval development, and reproductive capabilities. An enhanced egg production was measured in the individuals of F1 (19.65%). Additionally, an increased speed of development was detected for F2, which was confirmed by the increased amount of prepupae in F2 at day 10 of the experiment, compared to the different parental strains. Moreover, there was no significant difference in terms of bioconversion efficiency or feed conversion. This is interesting, as these outcomes can be reached in the F2 generation in a shorter amount of time due to an increased speed of development. We observed clear reciprocal differences, indicating that the choice of paternal and maternal lines is important. Therefore, this should be further explored when setting up crossbreeding schemes for BSF. For the total larval mass and dried larval mass production in F2, we calculated an increase of 16.5% and 19.1%, respectively, compared to the F0. These promising results suggest that crossbreeding could significantly boost the production capacity of BSF, making it a viable option for commercial rearing facilities. However, further research is necessary to fully understand the potential effects of heterosis in BSF and to develop strategies for implementing crossbreeding at an industrial scale.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.28194548

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Author contributions

Conceptualization: RM, LB, WG, SJ; formal analysis: RM, LB, JM; writing – original draft: RM, LB; writing – review and editing: RM, LB, JM, WG, LF, SJ, SVM, NB; funding acquisition: RM, SVM, NB; supervision: SVM, NB.

Conflict of interest

The authors have no conflict of interest to declare.

Data availability

The data generated and discussed in this research are available upon reasonable request with the authors.

Ethics

Ethical review and approval were waived for this study, as *Hermetia illucens* are not included in the European directive 2010/63/EU on the protection of animals used for scientific purposes.

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References

Adamaki-Sotiraki, C., Deruytter, D., Rumbos, C.I. and Athanassiou, C.G., 2023. Cross-breeding of *Tenebrio molitor* strains from a large-scale perspective. Journal of Insects as Food and Feed 10: 855-864. https://doi.org/10.1163/23524588-20230116

Amrul, N.F., Ahmad, I.K., Basri, N.E.A., Suja, F., Jalil, N.A.A. and Azman, N.A., 2022. A review of organic waste treatment using black soldier fly (*Hermetia illucens*). Sustainability 14: 4565. https://doi.org/10.3390/SU14084565

Ariyanto, D., Carman, O., Sulistyowati, D.T., Zairin, M., Syukur, M., Suharyanto, Himawan, Y. and Palimirmo, F.S., 2022. Analysis of combining ability and heterotic estimation on a complete Diallel cross involving five strains of common carp (*Cyprinus carpio*) from different geographical regions in West Java, Indonesia. Aquaculture Research 53: 6900-6909. https://doi.org/10.1111/ARE.16155

Barragan-Fonseca, K.B., Dicke, M. and van Loon, J.J.A., 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. Journal of Insects as Food and Feed 3: 105-120. https://doi.org/10.3920/JIFF2016.0055

Belperio, S., Cattaneo, A., Nannoni, E., Sardi, L., Martelli, G., Dabbou, S. and Meneguz, M., 2024. Assessing substrate utilization and bioconversion efficiency of black soldier fly (*Hermetia illucens*) larvae: effect of diet composition on growth and development temperature. Animals 14: 1340. https://doi.org/10.3390/ANI14091340

Booth, D.C. and Sheppard, C., 1984. Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing, and site characteristics. Environmental Entomology 13: 421-423. https://doi.org/10.1093/EE/13.2.421

Bosch, G., Oonincx, D.G.A.B., Jordan, H.R., Zhang, J., van Loon, J.J.A., van Huis, A. and Tomberlin, J.K., 2020. Standardisation of quantitative resource conversion studies with black soldier fly larvae. Journal of Insects as Food and Feed 6: 95-110. https://doi.org/10.3920/JIFF2019.0004

Broeckx, L., Frooninckx, L., Berrens, S., Wuyts, A., Sandrock, C. and Van Miert, S., 2022. Influence of strain genetics on larval performance and bioconversion efficiency for *Hermetia illucens*. Journal of Insects as Food and Feed 8: S50. https://doi.org/10.3920/JIFF2022.S1

Broeckx, L., Frooninckx, L., Slegers, L., Berrens, S., Noyens, I., Goossens, S., Verheyen, G., Wuyts, A. and Van Miert, S., 2021. Growth of black soldier fly larvae reared on organic side-streams. Sustainability 13: 12953. https://doi.org/10.3390/SU132312953

Broeckx, L., Frooninckx, L., Slegers, L., Berrens, S., Wuyts, A., Sandrock, C. and Van Miert, S., 2022. Influence of strain genetics on larval performance and bioconversion efficiency of *Hermetia illucens*. In: Annual Meeting of the European Federation of Animal Science (EAAP).

Broeckx, L., 2025. Genotype dependent digestion plasticity of black soldier fly larvae and its effect on larval performance and bioconversion efficiency. PhD Thesis, KU Leuven.

Cale, G.H. and Gowen, J.W., 1956. Heterosis in the honey bee (*Apis melifera* L.). Genetics 41: 292-303. https://doi.org/10.1093/GENETICS/41.2.292

Čičková, H., Newton, G.L., Lacy, R.C. and Kozánek, M., 2015. The use of fly larvae for organic waste treatment. Waste Management 35: 68-80. https://doi.org/10.1016/J.WASMAN.2014.09.026

DiGiacomo, K. and Leury, B.J., 2019. Insect meal: a future source of protein feed for pigs? Animal 13: 3022-3030. https://doi.org/10.1017/S1751731119001873

Dorper, A., Veldkamp, T. and Dicke, M., 2021. Use of black soldier fly and house fly in feed to promote sustainable poultry production. Journal of Insects as Food and Feed 7: 761-780. https://doi.org/10.3920/JIFF2020.0064

Ekechukwu, N.E., Baeshen, R., Traorè, S.F., Coulibaly, M., Diabate, A., Catteruccia, F. and Tripet, F., 2015. Heterosis increases fertility, fecundity, and survival of laboratory-produced F1 hybrid males of the malaria mosquito *Anopheles coluzzii*. G3: Genes, Genomes, Genetics 5: 2693-2709. https://doi.org/10.1534/G3.115.021436

Facchini, E., Shrestha, K., van den Boer, E., Junes, P., Sader, G., Peeters, K. and Schmitt, E., 2022. Long-term artificial selection for increased larval body weight of *Hermetia illucens* in industrial settings. Frontiers in Genetics 13: 865490. https://doi.org/10.3389/FGENE.2022.865490

Falconer, D.S. and Mackay, T.F.C., 1996. Introduction to quantitative henetics. 4th ed. Addison Wesley Longman, London, UK.

Frooninckx, L., Broeckx, L., Goossens, S., Wuyts, A. and Van Miert, S., 2024. Optimizing substrate moisture content for enhanced larval survival and growth performance in *Hermetia illucens*: exploring novel approaches. Discover Animals 1: 7. https://doi.org/10.1007/s44338-024-00005-2

Frooninckx, L., De Smet, J., Van Miert, S. and Van Der Borght, M., 2023. The need for dedicated pilot plant infrastructure for insect rearing and processing: a case-study from Belgium. Journal of Insects as Food and Feed 1: 5. https://doi.org/10.1163/23524588-20230032

Gasco, L., Biasato, I., Dabbou, S., Schiavone, A. and Gai, F., 2019. Animals fed insect-based diets: state-of-the-art on digestibility, performance and product quality. Animals 9: 170. https://doi.org/10.3390/ANI9040170

Gómez-Rubio, V., 2017. ggplot2 – Elegant graphics for data analysis (2nd edition). Journal of Statistical Software 77. https://doi.org/10.18637/jss.v077.b02

Guilliet, J., Baudouin, G., Pollet, N. and Filée, J., 2022. What complete mitochondrial genomes tell us about the evolutionary history of the black soldier fly, *Hermetia illucens*. BMC Ecology and Evolution 22: 72. https://doi.org/10.1186/S12862-022-02025-6

Hansen, L.S., Laursen, S.F., Bahrndorff, S., Kargo, M., Sørensen, J.G., Sahana, G., Nielsen, H.M. and Kristensen, T.N., 2024. Estimation of genetic parameters for the implementation of selective breeding in commercial insect production. Genetics Selection Evolution 56: 1-13. https://doi.org/10.1186/S12711-024-00894-7/TABLES/4

Henry, M., Gasco, L., Piccolo, G. and Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology 203: 1-22. https://doi.org/10.1016/J.ANIFEEDSCI.2015.03.001

Hoc, B., Noël, G., Carpentier, J., Francis, F. and Megido, R.C., 2019. Optimization of black soldier fly (*Hermetia illucens*) artificial reproduction. PLOS ONE 14: e0216160. https://doi.org/10.1371/JOURNAL.PONE.0216160

Hoecker, N., Keller, B., Piepho, H.P. and Hochholdinger, F., 2006. Manifestation of heterosis during early maize (*Zea mays* L.) root development. Theoretical and Applied

Genetics 112: 421-429. https://doi.org/10.1007/S00122-005-0139-4

Hull, K.L., Greenwood, M.P., Lloyd, M., Brink-Hull, M., Bester-van der Merwe, A.E. and Rhode, C., 2024. Drivers of genomic diversity and phenotypic development in early phases of domestication in *Hermetia illucens*. Insect Molecular Biology 33: 746-776. https://doi.org/10.1111/imb.12940

Jin, N., Liu, Y., Zhang, S., Sun, S., Wu, M., Dong, X., Tong, H., Xu, J., Zhou, H., Guan, S. and Xu, W., 2022. C/N-dependent element bioconversion efficiency and antimicrobial protein expression in food waste treatment by black soldier fly larvae. International Journal of Molecular Sciences 23: 5036. https://doi.org/10.3390/IJMS23095036

Kaya, C., Generalovic, T.N., Ståhls, G., Hauser, M., Samayoa, A.C., Nunes-Silva, C.G., Roxburgh, H., Wohlfahrt, J., Ewusie, E.A., Kenis, M., Hanboonsong, Y., Orozco, J., Carrejo, N., Nakamura, S., Gasco, L., Rojo, S., Tanga, C.M., Meier, R., Rhode, C. and Sandrock, C., 2021. Global population genetic structure and demographic trajectories of the black soldier fly, *Hermetia illucens*. BMC Biology 19: 1-22. https://doi.org/10.1186/S12915-021-01029-W

Lalander, C.H., Fidjeland, J., Diener, S., Eriksson, S. and Vinnerås, B., 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. Agronomy for Sustainable Development 35: 261-271. https://doi.org/10.1007/S13593-014-0235-4

Lan, L. and Nègre, N., 2023. Heterosis effect for larval performance of fall armyworm interstrain hybrids. Insect Science 31: 1296-1312. https://doi.org/10.1111/1744-7917.13295

Lippman, Z.B. and Zamir, D., 2007. Heterosis: revisiting the magic. Trends in Genetics 23: 60-66. https://doi.org/10.1016/J.TIG.2006.12.006

Makkar, H.P.S., Tran, G., Heuzé, V. and Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197: 1-33. https://doi.org/10.1016/J.ANIFEEDSCI.2014.07.008

Munsch-Masset, P., Labrousse, C., Beaugeard, L. and Bressac, C., 2023. The reproductive tract of the black soldier fly (*Hermetia illucens*) is highly differentiated and suggests adaptations to sexual selection. Entomologia Experimentalis et Applicata 171: 857-866. https://doi.org/10.1111/EEA.13358

Nelson, R.E. and Robison, O.W., 1976. Comparisons of specific two-and three-way crosses of swine. Journal of Animal Science 42: 1150-1157. https://doi.org/10.2527/jas1976.4251150x

Notter, D.R., Scherf, B. and Hoffmann, I., 2013. Breeding of animals. In: Levin, S.A. (ed.) Encyclopedia of biodiversity. 2nd ed. Academic Press, Cambridge, MA, USA, pp. 636-649. https://doi.org/10.1016/B978-0-12-384719-5.00016-2

Réale, D. and Roff, D.A., 2002. Quantitative genetics of oviposition behaviour and interactions among oviposition traits in the sand cricket. Animal Behaviour 64: 397-406. https://doi.org/10.1006/ANBE.2002.3084

Rhode, C., Badenhorst, R., Hull, K.L., Greenwood, M.P., Bester-van der Merwe, A.E., Andere, A.A., Picard, C.J. and Richards, C., 2020. Genetic and phenotypic consequences of early domestication in black soldier flies (*Hermetia illucens*). Animal Genetics 51: 752-762. https://doi.org/10.1111/AGE.12961

Ruban, P.S., Cunningham, E.P. and Sharp, P.M., 1988. Heterosis × nutrition interaction in *Drosophila melanogaster*. Theoretical and Applied Genetics 76: 136-142. https://doi.org/10.1007/BF00288844

Sandrock, C., Leupi, S., Wohlfahrt, J., Kaya, C., Heuel, M., Terranova, M., Blanckenhorn, W.U., Windisch, W., Kreuzer, M. and Leiber, F., 2022. Genotype-by-diet interactions for larval performance and body composition traits in the black soldier fly, *Hermetia illucens*. Insects 13: 424. https://doi.org/10.3390/INSECTS13050424

Siddiqui, S.A., Süfer, Ö., Çalışkan Koç, G., Lutuf, H., Rahayu, T., Castro-Muñoz, R. and Fernando, I., 2024. Enhancing the bioconversion rate and end products of black soldier fly (BSF) treatment – a comprehensive review. Environment, Development and Sustainability 2024: 1-69. https://doi.org/10.1007/S10668-023-04306-6

Ståhls, G., Meier, R., Sandrock, C., Hauser, M., Šašić Zorić, L., Laiho, E., Aracil, A., Doderović, J., Badenhorst, R., Unadirekkul, P., Mohd Adom, N.A.B., Wein, L., Richards, C., Tomberlin, J.K., Rojo, S., Veselić, S. and Parviainen, T., 2020. The puzzling mitochondrial phylogeography of the black soldier fly (*Hermetia illucens*), the commercially most important insect protein species. BMC Evolutionary Biology 20: 1-10. https://doi.org/10.1186/S12862-020-01627-2

Tomberlin, J.K. and Sheppard, D.C., 2002. Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. Journal of Entomological Science 37: 345-352. https://doi.org/10.18474/0749-8004-37.4.345

Tomberlin, J.K. and van Huis, A., 2020. Black soldier fly from pest to 'crown jewel' of the insects as feed industry: an historical perspective. Journal of Insects as Food and Feed 6: 1-4. https://doi.org/10.3920/JIFF2020.0003

Veldkamp, T. and Vernooij, A.G., 2021. Use of insect products in pig diets. Journal of Insects as Food and Feed 7: 781-794. https://doi.org/10.3920/JIFF2020.0091

Wiltbank, J.N., Gregory, K.E., Swiger, L.A., Ingalls, J.E., Rothlisberger, J.A. and Koch, R.M., 1966. Effects of heterosis on age and weight at puberty in beef heifers. Journal of Animal Science 25: 744-751. https://doi.org/10.2527/JAS1966.253744X

Yang, Z., Zhang, Z., Wang, G., Huang, Z. and You, W., 2024. Exploiting hybrid vigor to enhance survival and growth for whiteleg shrimp (*Litopenaeus vannamei*): s complete diallel cross experiment. Aquaculture and Fisheries (in press). https://doi.org/10.1016/J.AAF.2024.04.004