TITLE: Size-stabilized, Hypoallometric, Genitalia Determined for Male Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae)

RUNNING HEAD: Hypoallometric Genitalia in Black Soldier Fly

AUTHORS: *Lisa N. Rollinson[1,3], *Noah B. Lemke[1], Scott Crawford[2], James B. Woolley[1], and Jeffery K. Tomberlin[1]

* LNR and NBL are co-first authors.

AFFILIATIONS/ADDRESS

[1]Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX, USA

[2]Statistics Consulting Center, Texas A&M University, 3143 TAMU, College Station, TX, USA

[3]To whom correspondence should be addressed: Email: lisanrollinson@gmail.com

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Allometry, Reproduction, Morphology, Breeding

LAY SUMMARY:

Adult male black soldier fly shown to have similar sized genitals despite differences in body size.

SHORT SUMMARY (80 Words):

The black soldier fly, *Hermetia illucens,* is an economically important insect mass-reared throughout the globe; however, a large knowledge gap exists in terms of its reproductive physiology. This study examined the relationship of male genitalia to body size, finding a 10% increase in body size corresponded with a 1.8% increase in genitalia size, meaning the structures are hypoallometric. This finding is important because it indicates little adverse consequences for selecting larger flies, as differently-sized individuals should be able to copulate.

ABSTRACT:

The black soldier fly, *Hermetia illucens*, is mass-reared throughout the world to convert organic waste into ingredients for pet and livestock feed, as well as frass and other biological products. To promote the optimization of breeding regimes carried out by industrial black soldier fly production plants, it is critical to better understand adult reproductive physiology and its potential relationship with fertile egg production. However, in this species, little is known about how reproductive morphology changes with respect to increases in body size, nor the potential impacts selection can have on the form of reproductive structures. Hence, this study investigated the nutritional static allometric relationship between the external genitalia of adult male black soldier flies and their body size. Size differences were induced top-down by varying larval rearing densities, which has a downstream effect on adult body size. For each rearing density, a random sample of 30 individuals was selected, and measurements taken three times each for the head, thorax, parameral sheath, and gonostylus. Fitting a generalized linear log-log model to the data revealed that for every 10.0% increase in body size (thorax length), genital length (parameral shealth) only increased by 1.8%. The resulting allometric slope of genitalia to thorax size was 0.195, indicating a pattern of hypoallometry. The presence of hypoallometric genitalia in a domestic population indicates individuals can copulate irregardless of differences in body size, which is consistent with most other insects. Moreover, it is likely that the ancestral population of black soldier flies historically underwent selection to maintain a single genitalia-size independent of body size that continues to persist in captivity. To confirm, future work should investigate the direct impacts of hypoallometric genitalia on fitness, especially in flies which have been genetically edited or artificially selected to be increasingly large.

INTRODUCTION

The black soldier fly (BSF), *Hermetia illucens* (L. 1758) (Diptera: Stratiomyidae), is native to the southern United States as well as the American tropics (Kaya et al., 2021). It is bred industrially throughout the world for applications in animal feed and organic waste bioconversion. Over other insects, it is prized (Tomberlin & van Huis, 2020) not only for its ease of cultivation, but because its larvae can feed on most organic substrates and quickly bio-convert them into insect biomass and frass. Consider that in just a few weeks, BSF larvae can grow up to 10,000-times their body size, eating between 12.5 and 200-mg/day (Supriyatna et al., 2018; Dzepe et al., 2020). However, individual larvae are small, and so millions, if not billions, are needed to reach industrial targets, which can be as high as 60,000 metric tons of protein produced from waste conversion per year, or about 165 metric tons per day (C&E News, American Chemical Society). Companies have rallied around the promise of BSF in shaping future sustainable agricultural practices, and now billions of larvae are collectively produced per year across the globe (Barrett et al., 2023). But despite the fact that acquiring larvae relies on fertile egg production of the breeding colony, the reproductive biology of adult BSF remains understudied, especially compared to their larval counterparts (Lemke et al., 2023; Meneguz et al., 2023), as well as other mass-reared flies (Boller, 1972; Chambers, 1977; Scott et al., 2017). While the situation is not as critical as a decade ago (Čičková et al., 2015; Pastor et al., 2015), increased consistency and efficiency in breeding operations are both needed to reduce operational costs and drive down the final cost of industrial products (e.g., whole, dried, pulverized larvae, etc.). Therefore, this morphological study was designed as a first step to document the allometric relationships of male BSF genitalia of different size classes to aid ongoing optimization efforts of industrial breeding programs.

Historically, the focus of BSF-breeding programs has prioritized industrially relevant traits in larvae, such as increased larval mass, feed conversion ratios, or macronutrient profiles. Each of these has been accomplished both through artificial selection or genetic modification (Zhou et al., 2013; Zhan et al., 2020; Sandrock et al., 2022; Generalovic, 2023). Recent examples include an increase in size (Zhan et al.,

2020; Generalovic, 2023), differences in feed conversion rates (Zhou et al., 2013; Sandrock et al., 2022; Generalovic et al.), and increased larval nutrients such as carotenoids (Gunther et al., 2024) in industrial or research colony strains. Given these successes, greater attention should now be directed towards adult traits.

In other mass-reared insect species, such those reared for Sterile Insect Technique (SIT) (e.g., mosquitoes (Diptera: Culicidae), common fruit flies (Diptera: Drosophilidae), screwworm flies (Diptera: Calliphoridae), and true fruit flies (Diptera: Tephritidae)), high-priority traits include size, longevity, flight ability, and reproductive competitiveness (Lance & McInnis, 2021). In practice, however, translating such traits 1:1 to BSF is not necessarily needed and may in fact be contrary to industrial aims; for example, increased longevity would cause pre- and post-reproductives to overlap in cages using continuous release (Lemke et al. 2025). The ultimate fate of mass-reared BSF is not wild release, meaning artificial selection that reduces mating competitiveness in the wild does not need to be curbed. In practice this means, compared to SIT species, there are fewer adult parameters relevant for BSF production. Even so, with little species-specific knowledge of adult morphology, mating behavior, and sexual selection in BSF, it is difficult to know *a priori* how these interact to increase fertile egg production before they are first documented.

Certainly, increased larval size can translate to increased adult size and fecundity (Gobbi et al., 2013), but the interplay of adult morphology and life history traits with other industrial parameters is complex and not necessarily straightforward. Industrial producers typically operate by breeding large numbers of adults, often in cages as large as 6 m long (S. Salari, 2024). Densities within these cages can reach as high as 20,000 flies/m^3 (S. Salari, 2024). With such high densities, there are too many flies for the human eye to track, and densities even exceed the capabilities of computer vision or automated tracking systems, since classification models can break down whenever individuals leave and re-enter the camera frame (Nawoya et al., 2024). Previous studies, at greatly reduced spatio-temporal resolution, have examined stocking density (Hoc et al., 2019), heterogeneity of cohort size (Jones & Tomberlin, 2021) and age (Dickerson et al., 2024), as well as abiotic conditions (Li et al., 2024) and habitat design (Lemke et al.,

2024). To date, no studies have investigated the scaling of the external genitalia, which is almost certainly linked to pre- and post-copulatory processes and ultimately fitness.

Male adult BSF have external genitalia which grasp the female, allowing them to attach for ~30 min during mating (Giunti et al., 2018; Masse et al., 2023; Manas et al., 2025). Specifically, lateral extensions of the gonocoxite, which extend past the gonostylus, bend medially to grasp the female (Personal communication, Martin Hauser). The parameral sheath then slots into the female and the three aedeagal wands extend from the male's parameral sheath to release accessory fluid and sperm (personal observations). In addition, any of these male genital structures could play a role in mating success via postcopulatory female choice, since females might prefer certain shapes of genitals, which is the case for other flies (e.g., crane flies (Diptera: Tipulidae) (Eberhard & Gelhaus, 2009)), and these in turn might be affected by adult body size.

Black soldier fly genitalia structures may scale disproportionately with body size because the same is true for other anatomical structures. For example, BSF eye length, ommatidia density, and wing centroids do not scale proportionally with body size (Birrell, 2018). In addition, in laboratory settings, large flies, that have presumably received more nutrition as larvae, were found to have proportionally smaller eyes than those that received less nutrition as larvae because their eye size increased less than their body size (Birrell, 2018). Females exhibited larger antennae, wing centroids, and eyes, which may indicate sexual selection acting on these traits (Birrell, 2018). Put simply, large BSF are not scale models of small BSF.

In terms of reproductive structures, BSF genitalia are likely to be size-stabilized, meaning selection by females results in approximately one size of male genitalia irrespective of body size. Genital size stabilization is exhibited by most other insects and has been documented in the vast majority of analysed insect species (Eberhard, 2009). However, this pattern has yet to be confirmed in BSF. For these other insects, when larval nutrition is increased, adult body size in turn increases, and indeed this is also the case in BSF (Jones & Tomberlin, 2019). However, the rate at which genitalia size increases is typically much slower than for body size (Eberhard, 2009). This trend of nutritionally mediated static hypoallometry

122 (NMSH) is widespread, but not ubiquitous, in insects, and indeed three alternatives exist. When compared
123 to a smaller conspecific, an individual might have proportionally smaller, similar, or larger genitalia, which
124 are referred to as hypo-, iso-, or hyper allometry, and indicated when a log-log regression is fit to the data
125 and is either less than 1, statistically equal to 1, or greater than 1; respectively (Shingleton, 2010). Genitalia
126 with low allometric slopes have been theorized to increase the total number of females with which a given
127 male can mate and stimulate effectively (Eberhard, 2009). By contrast, hyperallometric genitalia are much
128 rarer but may still play a role in sexual selection. For example, male scorpionflies (Mecoptera), use their
129 genitalia directly in battles with rival males (Johnson, 1995; Eberhard, 2009).

130 The objective of this study was to investigate the NMSA relationship of male BSF genitalia using
131 morphometrics. Following the convention that isometry is used as a null hypothesis (Eberhard, 2009), we
132 present the null that male external genitalia in the BSF will scale in direct proportion (log-log slope=1) with
133 body size. However, our alternative hypothesis, we expect a hypoallometric deviation to occur (log-log
134 slope<1.00), following the general trend found in other insects (Eberhard, 2009). More broadly, this is a
135 step to understand how breeding amongst different sized flies might be maintained in the midst of industrial
136 selection pressures.

137

138 METHODS

139 Because the goal was to analyse genital allometry across a range of adult body sizes, and body size
140 is inversely proportional to the degree of larval competition in BSF (Jones & Tomberlin, 2019), larvae were
141 reared at three densities of 5,000, 7,500, and 10,000 per 10.5 kg of substrate. Critically, these sizes are
142 reasonable to have been sampled haphazardly from colony flies.

143 *Rearing Methods* - Eggs were harvested from a BSF colony established at the Forensic Laboratory for
144 Investigative Entomological Sciences (FLIES) at Texas A&M University. Larvae were reared on 70%
145 moisture Gainesville (Producers Cooperative Association, Bryan, TX, USA) diet (Hogsette, 1992)
146 according to the FLIES colony SOP (see: Supplementary Info) until they reached 8-d-old. After this, larvae

147 were split into different density treatments (n = 3 levels, n = 2 replicates) of approximately 5,000, 7,500,
148 and 10,000 larvae (gravimetrically estimated). Aliquots of larvae were placed directly atop 7.5 kg
149 Gainesville feed at 70% moisture in 27 L pans (Sterilite Corporation, Townsend, MA, USA). An additional
150 0.75 kg of dry Gainesville was placed around the edge of the pans to prevent escape. For the remainder of
151 the rearing-cycle, pans were housed in a walk-in incubator maintained at approximately 25°C, 50% relative
152 humidity, and 14:10 L:D photoperiod (fluorescent lighting).

153 Contents of each pan were hand-mixed on days 11 and 12 to vent heat, prevent anoxic composting,
154 and give larvae better access to the undigested portions of the substrate. An additional kilogram of 70%
155 moisture Gainesville diet was supplemented to pans on days 15, 16, and 18. On day 26, when at least 40%
156 of the larvae began pupation (Bosch et al., 2020), the two pans of larvae per density-level were combined
157 prior to being sieved. Then, 150 g aliquots from each respective density-level were transferred to cylindrical
158 plastic containers (13.5x13.2 cm: d × h) to pupate (Dickerson et al., 2024; Lemke et al., 2024). 150 g dry
159 Gainesville was used as bedding material, and the tops of the containers were secured with Tulle fabric
160 (Wal-Mart Stores Inc., Little Rock, AR, USA).

161 Newly emerged adults (unsexed) of each density level were collected daily from cylindrical
162 pupation containers and placed in BugDorm-1 rearing-cages (30 × 30 × 30 cm) (InsectaBio, Riverside, CA,
163 USA) housed within the same walk-in incubator for a minimum of 12 h, but not more than 36 h, so their
164 exoskeleton could sclerotize prior to being euthanized. During these procedures, mating was never observed
165 between flies within the Bug-Dorms, which aligns with established notions that mating occurs later in the
166 reproductive ontogeny (Tomberlin & Sheppard, 2002; Kobelski et al., 2024). The flies that emerged on
167 days 37-40, were then frozen in a -20°C freezer and transferred to 0.95-liter freezer bags for long-term
168 storage. Flies from each treatment level were combined in a 3.79-liter freezer bag and commingled.

169 *Sample Preparation* – Specimens were sorted based on external genitalia, and females discarded. Male
170 specimens from each treatment were selected on the basis that they did not have damage to their heads or
171 bodies. The sclerotized portion of the genitalia were removed whole and dry-mounted between two glass

172 slides. Unscleritorized tissue was removed with HTS 144S7 4.5” straight squeeze scissors (Hobby Tool
173 Supply USA LLC, Fallbrook CA, USA). The epandrium was also removed to improve the visibility of the
174 parameral sheath and gonostylus against the flat plane of the microscope slide. Dissected genitalia were
175 secured in square wells measuring approximately 4 × 4 mm created between two Corning 2947 Micro
176 Slides (Corning Glass Works, Corning, NY, USA) and two layers of Duck Brand masking tape (Shurtape
177 Technologies, LLC, Hickory, NC, USA). This allowed the genitalia to be stored without being crushed
178 (Supplementary Figure 1).

179 The head was removed from the thorax by cutting through the membranous neck. Each head was
180 mounted through the center, ventral to the antennae, using a #2 Physus pin (Making it Work Inc.,
181 Franklintown, PA, USA). Each thorax was pinned according to conventional standards, i.e., by placing a
182 #2 pin through the right side.

183 *Structures*- Because the parameral sheath is sclerotized, easily visible, and in direct contact with the female,
184 it was chosen as a good proxy for genital size. The gonostylus was chosen as a secondary proxy for genital
185 size because it less visible but is in contact with more of the genitalia and may help maintain its shape. This
186 investigation was limited to male external genitalia because female external genitalia are unsclerotized
187 (Munsch-Masset et al., 2023) and difficult to measure accurately; this is common in genital allometry
188 investigations.

189 *Measurements*- The length of the head, thorax, and parameral sheath were determined using a Leica M205
190 FA with a DFC 450 C lens operated with Leica LAS X software (Version 3.7.24914.5). These structures
191 were selected because they are sclerotized in BSF, and therefore putatively less prone to distortion from
192 mounting (personal observation, LNR). To account for error, each structure was measured on three separate
193 occasions. Between repeated measurements, the pinned head and thorax were moved from the specimen
194 stage and repositioned. Likewise, the genitalia structures were also moved from the specimen stage,
195 although they remained mounted in the wells of glass slides.

196 The length of each structure was automatically calculated using the integrated measure function
197 included in LAS X (Version 3.7.24914.5) by superimposing a line over the digital magnifications. How
198 these lines were drawn in reference to other anatomical reference points was defined by Birrell (2018),
199 though we note that in their work head length and thorax length are instead referred to as eye length and
200 pronotum length [sic].

201 Per Birrell (2018), head length was defined as the distance from the dorsal to ventral boundary of
202 the inner margin of the right compound eye, and thorax length was taken from the anterior of the pronotum
203 to the posterior of the scutellum. The parameral sheath was measured medially, perpendicular to its
204 intersection into the rest of the genitalic structure. The length of the gonostylus was taken from the distal
205 tip to a line perpendicular to the base of the parameral sheath (Figure 1).

206 *Statistical Analyses* - Raw data was cleaned in MS Excel (Version 2308 Build 16731.20170) by LNR to
207 indicate damaged specimens (n = 4) and later analyzed using R Studio (Version 4.2.2) by SC at the Texas
208 A&M Statistical Consulting Center. For each of the three rearing density levels, 30 flies were measured,
209 less the damaged specimens, yielding 86 total replicates (5,000 n = 28, 7,500 n = 29, 10,000 n = 29) (Table
210 1).

211 Because each fly replicate was re-measured three times, a model needed to be devised that could
212 account for measurement error. A generalized linear log-log model with random effect for 'fly' using
213 backwards variable selection to model *P*-values based on Satterthwaite degrees of freedom with Kenward-
214 Roger approximation was chosen (which we will abbreviate as a 'random effects model'). Alpha for the
215 analyses was set at 0.05.

216 *Model Selection*- Although preliminary results comparing untransformed data to a log-log transformation
217 were similar, the latter was preferred to be consistent with allometric Power-Scaling Laws seen in the
218 general literature (Shingleton et al., 2007). Preliminary modelling also revealed that thorax and head lengths
219 were highly positively correlated ($R^2 = 0.90$), and statistically significant ($P = 2.2*10^{-16}$), with a quadratic

interaction between increasing length of parameral sheath and larval density. Still, larval density was removed from the final model because it is an expected artifact of nutrition affecting body size (Jones & Tomberlin, 2019). To simplify the model further, the random effects model only considered the effects of thorax on genitalia (parameral sheath, gonostylus). Head size was omitted because thorax length is a more standard proxy for body size.

Dimensional Reduction - In addition to the random effects model, principal component analysis (PCA) was performed (Borcard et al., 2011) on the untransformed data to explore how thorax, head, sheath, gonostylus, and larval density were related to one another in multidimensional space. (Supplementary Figure 2) Because much of the variability could be explained by the first two principal components (>75%), only these were retained to make model interpretation tractable (Supplementary Figure 3; Supplementary Table 1).

RESULTS

Chosen Model- The generalized linear log-log model with backwards variable selection found that the slope of the logarithmic line, α, was highly significantly different from 1 (two-tailed $|P| < 0.006$, df = 193.74). Therefore, the null hypothesis of isometry (slope=1) was rejected (Table 2).

The random effects model produced the following equation:

$$\ln(\text{Sheath}) = -7.61413 + 0.19468 \ln(\text{Thorax})$$

On a log-log plot, this equation appeared as a straight line with a slope (α) of 0.19468 and a y-intercept of -7.61413 (Figure 2).

A 10% increase in the size of the thorax was shown to translate to a 1.87% increase in sheath size. Some sample projections are provided in Table 3. Study data support a 10% increase in size. Values greater than this were extrapolated for achievable increases on body size based on industry press releases (Varol, 2023) and artificial selection studies (Generalovic Thesis).

Principal Component Analysis (PCA) indicated most of the variance in density, thorax, and head were explained by principal component one, while the variance for gonostylus and sheath are almost solely

244 explained by principal component two. Sheath length had a slight positive association with principal
245 component two, indicating its correlation with head and thorax length. In addition, PCA indicated that
246 thorax and head are closely related, with an inverse relationship to density.
247
248 DISCUSSION

249 The aim of this study was to document the nutritionally mediated static allometry of adult male
250 BSF external genitalia. The data collected indicate that the log-log relationship between adult BSF thorax
251 length and male parameral sheath length significantly deviated from 1.0. Thus, the null hypothesis of
252 isometry was rejected, and the alternative confirmed: male BSF genitalia were found to be hypoallometric
253 with respect to body size ($P = 0.005$), which aligns with patterns found in most other insects studied thus
254 far. The models developed in this study specifically indicate a nutritionally mediated 10% increase in thorax
255 length, the proxy for body size, correlated with just a 1.87% increase in parameral sheath length, the proxy
256 for genitalia size. Effectively, this means both large and small BSF from the same research colony have
257 similarly sized genitalia relative to their body size. These findings affirm that while many aspects of adult
258 BSF reproduction are size-dependent, external male genital morphology is relatively size-independent; this
259 could be relevant to industrial producers seeking to simplify their selection regimes by reducing the number
260 of parameters being considered for their breeding programs. Still, although only a 5.87% proportional
261 increase in genitalia relative to body size was observed (10% thorax divided by 1.87% parameral sheath),
262 insects may be sensitive to even small changes, being small themselves. Future studies linking genital size
263 increases directly with fitness are needed to confirm.

264 Indeed, in other insect species, the size and shape of genitalia has been demonstrated to affect
265 reproductive success. Although sexual selection on genitalia is difficult to directly quantify (Eberhard,
266 2011), larger genital size has been positively correlated with paternity rates and negatively associated with
267 sperm rejection in tortoise beetles (Coleoptera: Chrysomelidae) (Rodríguez et al., 2004). In addition,
268 reducing male genitalia size through ablation was shown to alternatively reduce reproductive success in a

seed bug (Hemiptera: Lygaeidae) (Dougherty et al., 2015), increases female sperm rejection in a tortoise beetle (Coleoptera: Chrysomelidae) (Rodriguez, 1995), and can reduce copulatory success in fruit flies (Diptera: Drosophilidae) (Grieshop & Polak, 2012). Likewise, altering the scaling of insect genitalia through genetic modifications can also negatively impact reproductive success. For instance, *Drosophila melanogaster* Meigan 1830 (Diptera: Drosophilidae) genitalia size have been experimentally reduced by genetically editing the flies to make Forkhead box O (FOXO) activity, which slows growth when nutrition is low, constitutively active. Males with posterior genital lobes that were smaller were less likely to copulate and produced fewer offspring (Dreyer & Shingleton, 2011). These studies hint that the maintenance of appropriately sized hypoallometric genitalia could be important to the reproductive success of many insects, possibly including black soldier flies.

Extrapolating further, the hypoallometic genitalia of BSF may be a result of similar selective pressures as in other insects and may be similarly important in reproductive success. In insects broadly (Eberhard, 2009) past selection events have acted on genital structures to maintain hypoallometric relationships, and the same may be occurring in BSF, though this is unconfirmed. Theory indicates that over evolutionary time, a single optimal genital size could have been achieved through three alternative routes: (1) stabilizing selection (Eberhard et al., 1998), (2) two opposing forms of directional selection (Dreyer & Shingleton, 2011), or (3) different strengths of selection on different sizes of flies (Eberhard et al., 2009). In terms of reproductive ecology, adult female BSF may have had exhibited either a choice for a single preferred size of male genitalia (Eberhard et al., 1998) or two co-occurring mating strategies that selected for smaller genitalia in large flies and larger genitalia in small flies (Eberhard et al., 2009; Dreyer & Shingleton, 2011). Preserved over time, either of these cases could result in a single preferred size of male genitalia and thus a hypoallometric relationship, as observed in the flies of our study. In other species, this choice for genital size has been postulated to be driven by increased stimulation and is not necessarily based on a precise mechanical ‘locking’ fit because the female genitalia are flexible and yielding (Eberhard et al., 1998; Munsch-Masset et al., 2023).

LIMITATIONS

This study was limited to linear measurements of external adult male genitalia in a single population of BSF and offers a single, preliminary data set to guide future research. Linear measurements of the thorax, head, and genitalia may not fully explain the three-dimensional changes in morphology and may be subject to measurement error, but the use of multiple structures and a model incorporating repeated measurements was used to partially mitigate this. As in many genital allometry studies, female structures were not measured due to their non-sclerotized nature, although attempts were made. Additionally, the nature of purely morphological data limits predictions of sexual selection and reproductive success related to BSF genital size, especially across populations and strains. The allometric slope of male genitalia has also been shown to differ across populations (Kawano, 2002; Bertin & Fairbairn, 2007; Eberhard et al., 2009) and may do so in BSF. The exact genetic provenance of this population is not fully known, although the EVO Conversion Systems strain descended from the original Sheppard strain, which has served for numerous populations globally.

As in many genital allometry studies, female structures were not measured due to their non-sclerotized nature, although attempts were made. The nature of purely morphological data limits predictions of sexual selection and reproductive success related to BSF genital size, especially across populations and strains. The allometric slope of male genitalia has also been shown to differ across populations (Kawano, 2002; Bertin & Fairbairn, 2007; Eberhard et al., 2009) and may do so in BSF. The shifts in mating systems that BSF have undergone in industry may have shifted the exact allometric relationship, however determining differences between populations is beyond the breadth of the current study. Given the ubiquity of hypoallometry in insects (Eberhard et al., 1998), theories of selection maintaining hypoallometric genitalia (Eberhard et al., 1998), and the mechanisms by which sexual selection acts on genital size in other insects (Dreyer & Shingleton, 2019), it is probable that this hypoallometry is consistent across different BSF strains.

FUTURE DIRECTIONS

This investigation acts as preliminary research documenting the allometric relationships in male BSF external genitalia. Future studies should investigate the direct relationship between size-stabilized genitalia on fitness by examining how increases in body size and corresponding increases in genital parameters act to influence mating success and fertile egg production. Differences in adult mass of just 50% have been shown to increase hatch variability by 40 times (Jones & Tomberlin, 2021); however, the reason for this variability has yet to be determined, and could be due to physiological or behavioral mechanisms, or some combination of the two. Examining the behavior, egg production, and hatch rate of individual mating pairs of different sizes could elucidate the reason for hatch variability in heterogeneous populations.

In addition, future research should consider using genetically modified strains, so that the results can be better generalized to industry, where improvement through traditional selection and genome modification can increase mean pupal body size by 26% (Generalovic Thesis) and 50% (Zhan et al., 2020; Varol, 2023) respectively, with similar numbers being reported in industry press releases. Genetic editing, especially of insulin pathways, could theoretically impact allometric relationships as well, and impact mating success. However, over time natural selection could reinforce effective genitalia sizes and allometric relationships in the edited population. Hence, understanding how artificial selection and gene-editing interplay to affect adult morphology and reproductive success will be important for understanding the dynamics of sexual selection during mass-breeding.

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CONFLICT OF INTEREST

Material for use in this project was purchased from EVO Conversion Systems LLC, a company with which Dr. Tomberlin has a significant financial interest. This conflict of interest is managed by a plan submitted to and approved by Texas A&M University and Texas A&M AgriLife.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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FIGURES

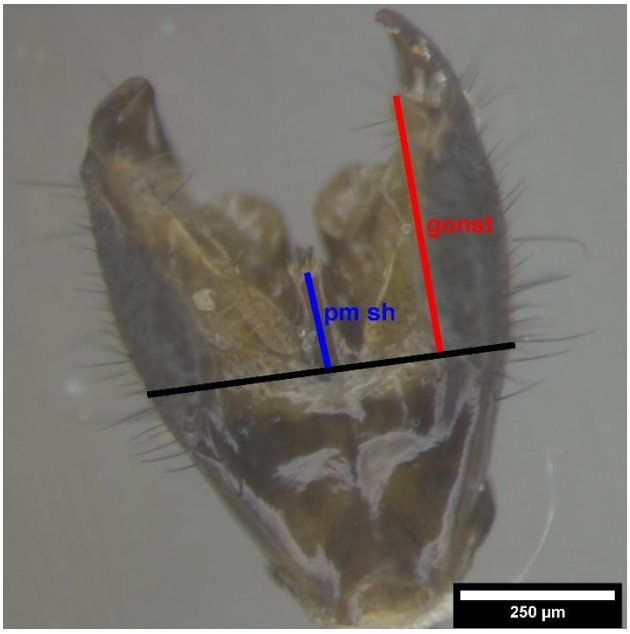
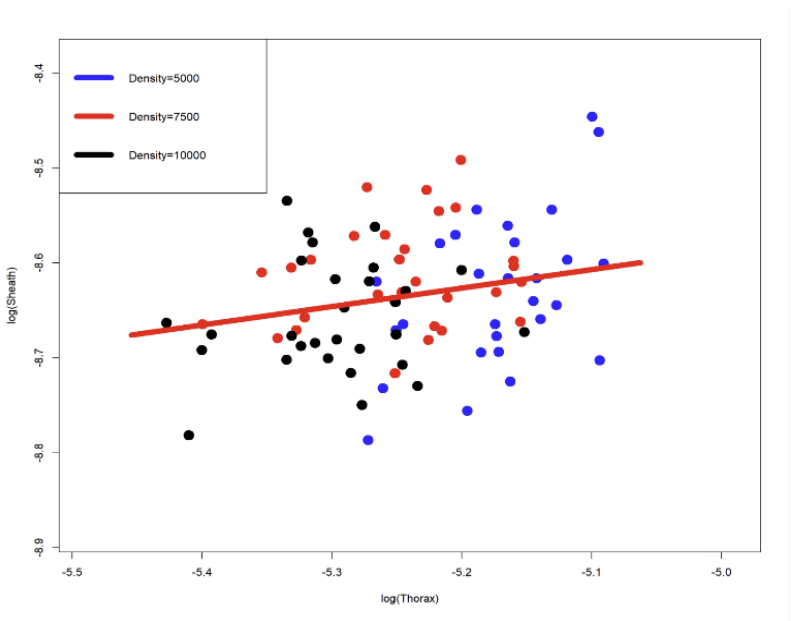


Figure 1. Photo of male black soldier fly genitalia with epandrium already removed to increase visibility to the structures below. Colored measurement lines incidate the parameral sheath (pm sh) in blue and gonostylus (gonst) in red.



535 Figure 2. Black soldier fly sheath length plotted against thorax length, with both axes log transformed. Data
536 points are colored according to the larval rearing density (i.e., 10 000, 7 500, or 5 000 larvae/pan), and each
537 point represents the average for each individual specimen (n = 30), which was measured three times.
538
539
540 TABLES

541 Table 1. Black soldier fly body and genitalia measurements (mm). Thorax and head lengths were used as
542 proxies for body size, whereas the gonostylus and shealth were measured as proxies of genital size.
543 Presented below are the group means ± SD and ranges (min, max) for these measurements according to
544 each density that specimens were reared at (10 000, 7 500, or 5000 larvae per container). Treatment groups
545 were pooled together to yield the grand mean and total range.

	10,000		7,500		5,000		Combined	
	Mean ± SD	(Min,Max)	Mean ± SD	(Min,Max)	Mean ± SD	(Min,Max)	Mean ± SD	(Min,Max)
Thorax	5.012 ± 0.313	(4.278, 5.856)	5.262 ± 0.34	(4.433, 5.86)	5.678 ± 0.304	(5.067, 6.328)	5.313 ± 0.42	(4.278, 6.328)
Head	2.072 ± 0.094	(1.871, 2.318)	2.147 ± 0.098	(1.954, 2.304)	2.266 ± 0.077	(2.106, 2.433)	2.16 ± 0.12	(1.871, 2.433)
Gonostylus	0.391 ± 0.024	(0.345, 0.454)	0.401 ± 0.023	(0.349, 0.448)	0.388 ± 0.032	(0.315, 0.438)	0.393 ± 0.027	(0.315, 0.454)
Sheath	0.174 ± 0.011	(0.142, 0.199)	0.182 ± 0.011	(0.161, 0.207)	0.179 ± 0.015	(0.152, 0.221)	0.178 ± 0.013	(0.142, 0.221)

546 Table 2. Coefficients table. A generalized linear log-log model with backwards variable selection relating
547 increases in adult male BSF genital size (parameral sheath) to increases in body size (Thorax).

	Estimate	Std. Error	df	T value	Pr(>|t|)
(Intercept)	-7.61413	0.36231	193.69667	-21.016	< 0.0000 ***
log(Thorax)	0.19468	0.06912	193.74983	2.817	0.00536 **

548

549 Table 3. Estimated projections for the absolute increase in male genital (sheath) size with respect to
550 increases in body (thorax) size in the black soldier fly. Body size increases of 10% or less are interpolated

551 from this study's data. Beyond this range, the projected size increase to genitalia is extrapolated from other

552 studies or claims made by industry press releases.

Increase in Body Size	**Reference**	**Increase in Genital Size**
+5%	Interpolation	0.95%
+10%	Interpolation	1.87%
+25%	Artificial Selection (Generalovic Thesis)	4.44%
+50%	FreezeM Genetic Line (Varol, 2023)	8.21%
+100%	Extrapolation	14.4%

553

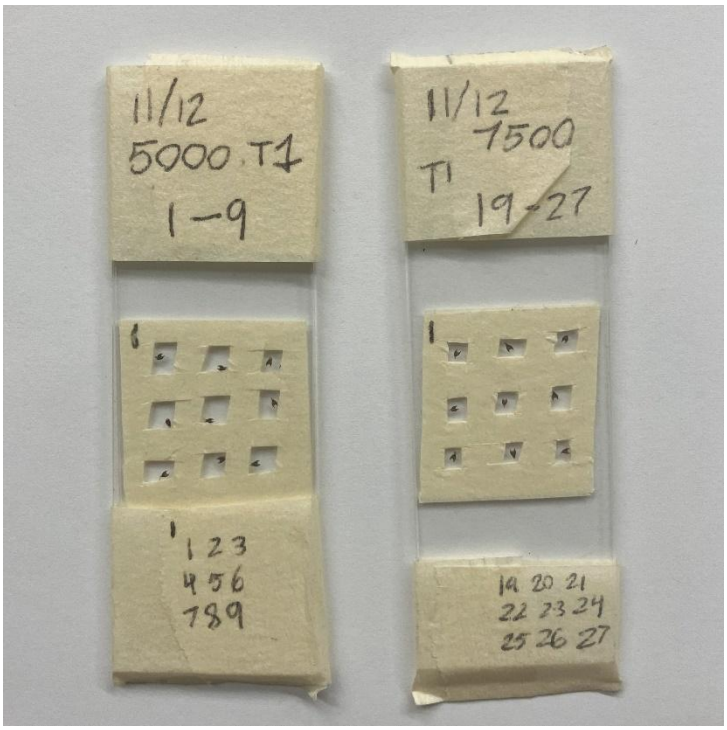
554

SUPPLEMENTARY INFO

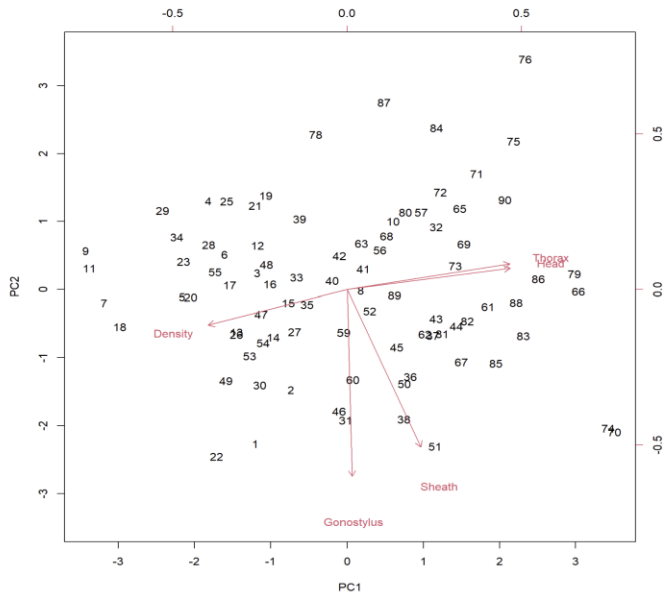
FLIES Colony SOP

Oviposition traps of fluted cardboard were collected from the colony and transferred into a mason jar covered by two layers of paper towel to allow airflow but prevent escape. During this time, the eggs hatched, and neonates migrated into the bottom of the mason jar. Then, 0.5 g of neonates were transferred into each of three square plastic containers. The neonates were fed with 500 g Gainesville feed (Hogsette 1992) formulated at 70% moisture. A border with 50 g of dry feed was used to prevent larvae from escaping. The black soldier fly larvae were reared in a walk-in incubator at approximately 27 °C.

Supplemental Figure 1. Genitalia secured between microslides in wells made of two layers of Duck Brand masking tape.



Supplemental Figure 2. Principal component analysis (PCA) performed with the variables of thorax, head, sheath, gonostylus, and larval density



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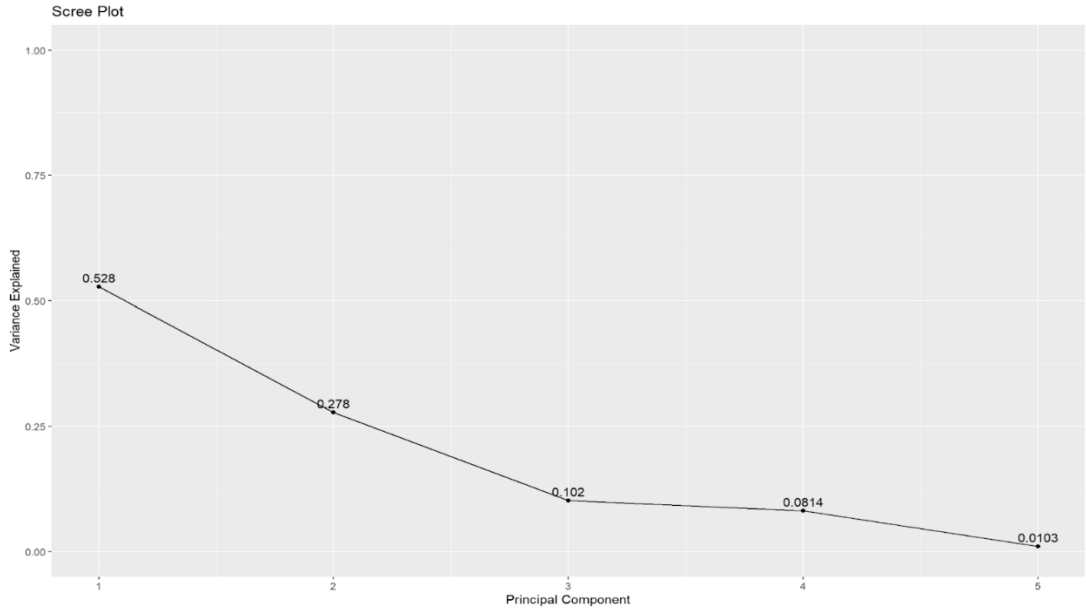
571 Supplemental Figure 3. Scree plot demonstrating that principal components and two explain a sufficient

572 amount of the variability (>75%).



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574

575 Supplemental Table 1. Principal Component Analysis

	PC1	**PC2**

Thorax	0.58361004	0.1027914
Head	0.58406062	0.0847625
Sheath	0.26451689	-0.6319838
Gonostylus	0.01790199	-0.7499736
Density	-0.49797876	-0.1427769

576