Body Mass Increase Induced by Eight Years of Artificial Selection in the Yellow Mealworm (Coleoptera: Tenebrionidae) and Life History Trade-offs

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Abstract

Efforts to improve rearing conditions of Tenebrio molitor L. (Coleoptera: Tenebrionidae) for insect biomass production included selecting for larger size pupae. The effects of an 8-yr continuous selection of T. molitor pupae for larger size were studied. Data consisting of daily counts and weights of pupae were analyzed using regression to determine the effects of selection over time. A preliminary evaluation of food conversion, growth, fecundity, and larval survival was done to compare ancestral versus selected strains. A significant positive correlation was identified between pupal size and time indicating a significant increase in pupal size over time in the selected T. molitor strain. A preliminary comparison of ancestral and selected strains showed significantly larger pupal size, growth rate, fecundity, and efficiency of conversion of ingested food in the selected strain. However, the selected strain also showed significantly lower larval survival than the ancestral strain. The low larval survival impacted the overall productivity of the selected strain resulting in no significant differences in biomass production when compared with the ancestral strain. The potential of using selection to improve biomass productivity in T. molitor is discussed.

Key words: insect rearing, biomass production, food conversion, insects as feed and food, fecundity

The yellow mealworm, Tenebrio molitor L. (Coleoptera: Tenebrionidae) is one of the few species of insects that is mass produced commercially (Heckmann et al. 2018) for purposes other than supporting sterile male release programs. Commercial production of T. molitor in the United States started in the 1950s and it was sold as fish bait through the 1970s. Mealworms including T. molitor were introduced into the exotic pet food market in the late 1970s and this industry is currently well established in the United States with dozens of producers (Martin et al. 1976, van Huis et al. 2013, Makkar et al. 2014, Shockley and Dossey 2014, Cortes Ortiz et al. 2016, Dossey et al. 2016, Shockley et al. 2018).

Insects are emerging as a valuable new crop for food security. Insects and mealworms in particular, have great potential as animal feed as established in multiple studies (Martin et al. 1976, Ng et al. 2001, Ramos-Elorduy et al. 2002, van Huis et al. 2013, Barroso et al. 2014, Makkar et al. 2014, Belforti et al. 2015, de Marco et al. 2015, Sánchez-Muros et al. 2016). Tenebrio molitor has been identified as a potential replacement for unsustainable sources of animal protein in feed formulations (Barroso et al. 2014, Bosch et al. 2014, Sánchez-Muros et al. 2016, Fanatico et al. 2018). The inclusion of up to 25% T. molitor in the diet for European bass (Dicentrarchus labrax L.) (Perciformes: Moronidae) juveniles showed promising results as a replacement of fish meal (Gasco et al. 2016). Higher percentages of T. molitor, however, resulted in lower food intake and lower weight gain in African catfish (Clarias gariepinus Burchell) (Siluriformes: Clariidae) (Ng et al. 2001), European bass (Gasco et al. 2016), and rainbow trout (Oncorhynchus mykiss (Walnaum)) (Belforti et al. 2015), probably due to the higher fat content of T. molitor (Barroso et al. 2014, Belforti et al. 2015). Sánchez-Muros et al. (2016) suggested the use of defatted T. molitor paste in feed formulations for fish as a way to reduce the lipid and increase the protein content. The resulting oil can also be used as a feed ingredient, or in the production of biodiesel (Manzano-Aguiliero et al. 2012).

Insects hold an important role in the future of food security and sustainability (Gahukar 2011, 2016; Pal and Roy 2014; van Huis et al. 2015; Dunkel and Payne 2016; van Huis and Dunkel 2016) and T. molitor has been identified as a novel source of human food (DeFoliart 1999, Aguilar-Miranda et al. 2002, Ramos-Elorduy 2009, Gahukar 2011, van Huis et al. 2013, Shockley and Dossey 2014, van Huis 2017). The larvae of T. molitor contain adequate nutrition for maintaining human health (Barker et al. 1998; Finke 2002, 2015; Finke and Oonincx 2014). Payne et al. (2016) graded T. molitor as significantly healthier than beef or chicken using two nutrient...
profiling models for human nutrition. In addition, the environmental impact of mass producing *T. molitor* is lower compared with other sources of animal food (Ramos-Ellorduy 1997, Gahukar 2011, Oonincx and de Boer 2012, Halloran et al. 2016), especially when considering the emissions of greenhouse gases (Oonincx et al. 2010). Moreover, *T. molitor* production requires less space and water than production of other animals (Oonincx and de Boer 2012, Gahukar 2016, Halloran et al. 2018). In their life-cycle assessment, Smetana et al. (2016) concluded that the production of insects using agricultural by-products is 2–5 times less environmentally destructive than the production of traditional meat products.

Despite the potential of *T. molitor* as a nutritious and sustainable feed or food, the methods for mass producing insect biomass remain primitive and inefficient, requiring high levels of manual labor (Cortes Ortiz et al. 2016). Deficiencies in the current production systems result in poor economics for food production, keeping mealworms too expensive for agricultural uses. In addition to suboptimal mass production technology, commercial production of *T. molitor* currently relies on a diverse number of strains with undocumented characteristics, which rarely have been selected for optimized biomass production. There has been virtually no selection on the species for desirable traits, a key element of any agricultural crop production system, and an important step in bringing down the costs of production. Previous work has shown that optimization of biomass production can be achieved in *T. molitor* by selecting for simple characteristics such as pupal size (Leclercq 1963).

This work presents results from a continuous effort to improve the rearing conditions of *T. molitor* by implementing better practices supported by experimental data over the past 8 yr. The objectives of this study were to determine whether the average pupal size of *T. molitor* had increased significantly after 8 yr of artificial selection and to investigate the effects of this selection on food conversion, growth, fecundity, and larval survival of *T. molitor*.

**Materials and Methods**

**Colony Maintenance**

The *T. molitor* colony used in this study was originally established in 2005 from stock donated by Southeastern Insectaries Inc. (Perry, GA), and it has been continuously grown at the National Biological Control Laboratory (Stoneville, MS) for the last 13 yr. The colony was maintained in a rearing system consisting of stacked fiberglass trays (MFG Tray Co., Linesville, PA) with screened bottoms (500 µm pore size) to grow the larvae and boxes with screened bottoms (850 µm pore size) to hold the adults (oviposition boxes). Collection of first instars was done in a second tray underneath adult trays (Morales-Ramos et al. 2012, Cortes Ortiz et al. 2016, Shapiro-Ilan et al. 2016). Larvae were fed mostly with wheat bran (>90%) and supplemented with 5–10% dry potato squares once a week. Adults were fed with 90% wheat bran and 10% of a supplement consisting of 84% dry potato, 12% dry egg white, 2% soy protein, and 2% peanut oil. Adults were misted with water twice a week with a spray bottle. Because larvae of *T. molitor* have the ability to take up water in subsaturated air (Dunbar and Watson 1975), the relative humidity in the rearing room was maintained at 70% or higher and larvae were not provided with water. The room was kept under dark conditions at 26°C.

**Larval Collection and Development**

New progeny for colony reproduction was obtained using a modified two-tray stacked system (oviposition boxes). The top trays were stackable fiberglass trays 59.37 × 30.48 × 15.24 cm (long by wide by deep) with lid and the bottom trays were stackable fiberglass trays 59.37 × 30.48 × 7.37 cm (long by wide by deep). The top tray was modified by replacing the bottom with nylon screen standard No. 20 (with 850 µm openings) and by adding three circular screened windows (5.5 cm diam.) along both of the long sides of the tray. Adults oviposited and glued the eggs to the bran flakes and sides of the top tray. Eclosed first instars fell through the screen openings to the bottom tray.

First instars were collected weekly from the bottom trays of oviposition boxes and placed in plastic boxes (950 ml, 220 × 150 × 52 mm) where they remained for a period of 30 d to allow them to grow larger than the screen openings of the next set of trays. After 30 d, the weekly cohort was transferred to a plastic box 24 × 35 × 12.5 cm modified by replacing the bottom with nylon screen (standard No. 35 with 500 µm openings). Frass particles falling through the 500 µm mesh were collected in an unmodified box at the bottom. The frass collected in the bottom box was weighed after 5 wk. The weight of the frass produced in a 5-wk period was used to estimate larval density based on a preliminary test with groups of 100 larvae. The number of larvae in the box was estimated by multiplying the frass weight in mg by a constant (9.26258) obtained in the preliminary test. This crude method was used to control larval density in the large larval trays.

Larger larvae were transferred to the stacked larval tray system, which consisted of Stackable fiberglass trays 65.4 × 45.08 × 15.24 cm (long by wide by deep) modified by replacing the bottoms with nylon screen standard No. 35 (500 µm) and adding seven circular screened windows in each of the longer sides and three in each of the shorter sides for ventilation. Larval density in the trays was limited to a maximum of 7,000 and a maximum of 14,000 larvae. Larval trays were stacked in groups of up to five trays. Frass moved from the top tray to the bottom passing through all the intermediate trays aided by the continuous movement of the larvae to an unmodified tray of the same dimensions located at the bottom of the stack for frass collection.

Larvae were separated by size when they reached 5 mo of age using a three-screen circular-motion vibrating separator (Midwest Industries Inc., Macon, GA) that divided larvae into three groups according to their size. The openings of the three screens were rectangular and measured 12.7 × 1.854, 12.7 × 1.65, and 12.7 × 1.09 mm. The first group of larvae that could not pass through the larger screen openings (12th instar or older) was transferred to plastic trays (52 × 39.5 × 12 cm) having solid bottoms (with no screen) provided with food and allowed to pupate. The second group of smaller larvae (11th and 10th instars) were placed in new screen bottom trays as described earlier in this subsection (grouped by size), provided with food to continue development. Larvae from the third group, consisting of much smaller larvae, were discarded as one of the quality control protocols. These larvae displayed an extreme delay in development that may signal deteriorated health.

**Colony Reproduction**

Pupae were collected daily from the unscreened larvae trays by using a sifter standard No. 5 (4 mm openings). Daily pupae collections were stored in an environmental chamber at 15°C for a maximum period of 6 wk. The total number of pupae collected and their total weight was recorded daily in the colony log. The mean pupal weight was calculated as the total weight divided by the number of pupae.

Pupae for reproduction were selected from the stored pupae. Selected pupae were allowed to complete development in a plastic tray with a solid bottom lined with tissue paper at the rearing conditions described in the “larval Collection and Development” subsection. Emerging adults were kept in the emergence tray for 3–4 d until...
most of them attained a black or dark brown coloration. Mis-molted and deformed adults were discarded. Healthy adult beetles (250–300 individuals, 1:1 sex ratio) were transferred to oviposition boxes as described in the “Larval Collection and Development” subsection. Sex ratio was determined to be a consistent 1:1: male after 2 yr of counts using the method of Bhattacharya et al. (1970) of sex determination for pupae and adults. Adults were provided with 400 g of wheat bran and 30 g of adult supplement (described subsequently) in each box. The food provided is sufficient to maintain the adults for the 8-wk reproductive period and additional feeding is not required.

**Adult Supplement Preparation**

A 500 g of dry supplement was prepared by mixing 420 g of dry potato flour, 60 g of dry egg white, 10 g of soy protein, and 10 g of peanut oil in a stainless steel bowl using spatula and 700 ml of reverse osmosis (RO) water were added to the mix. The formulation was then mixed using an electric blender for 20 min. Portions of the mixed supplement were dispensed into the cavities of a cookie pan using an ice cream scoop (60 ml). Supplement portions were dried in a vacuum oven at 50°C and 100 mbar vacuum for a period of 48 h. The dry supplement form pellets weighing approximately 27 g each. Dried supplement pellets can be stored for more than 1 yr if kept dry.

**Reproductive Adult Colony History**

Between January 2007 and April 2008 groups of up to 1,000 adults were introduced to each oviposition box in a continuous way as they became available from developing pupae. During these period, oviposition boxes consisted of two stacked Rubbermaid containers divided by a screen as described in the “Larval Collection and Development” subsection. After June 2008, quality control protocols were introduced, including eliminating damaged or deformed pupae and mis-molted adults. Also, adult densities in the oviposition boxes were reduced to 700 adults per box. Studies on reproductive biology of *T. molitor* showed that adult density impacts significantly the production of progeny per unit of rearing surface (Morales-Ramos et al. 2012). Based on this research, adult densities in the reproductive colony were substantially reduced. In March 2010, the two-tray system for oviposition boxes described in the “Larval Collection and Development” subsection was introduced to the colony, the density of adults per oviposition box was reduced to 500 and selection for larger pupal size was initiated. In addition, adults older than 9 wk were discarded after research showed that 90% of the potential reproduction occurred in the first 9 wk of adult life (Morales-Ramos et al. 2012). In September 2011, adult density in the oviposition boxes was reduced to 250 and adults were discarded after 8 wk of oviposition. In July 2012, a supplement was added to the adult diet to increase fecundity and reproductive longevity based on Morales-Ramos et al. (2013). In January 2014, reproductive grouping started by storing pupae at 15°C to synchronize development. This allowed for the maintenance of adults cohorts of approximately the same age and to regularize reproductive cycles. Pupae were stored for up to 8 wk at this temperature, but increasing numbers of deformities and adult molting defects became a problem at times when the pupae used had been stored for longer than three weeks. Starting in November 2016, the storing time of pupae was limited to 2 wk.

**Larval Growth Rearing System History**

From January 2007 to February 2010, larvae were grown in nonstackable rubber made boxes 24 × 35 × 12.5 cm modified with screened bottoms as described in the “larval Collection and Development” subsection. Larval densities in the boxes were unknown and highly variable, depending mostly on the adult oviposition levels. The stackable tray system described in the “Larval Collection and Development” subsection was introduced in March 2010, but larval densities in the trays remained variable until October 2015. A crisis occurred in the main colony during the entire year of 2012. Larvae mortality increased to over 50% due to fungal infection by *Aspergillus flavus* and *A. fumigatus*. During this period, an unrecognized humidifier malfunction occurred reducing RH to 50%. During 2012, larval densities in the trays increased due to improved progeny production as a result of implemented improvements in adult rearing conditions. Later studies established the impact of larval density on growth and survival (Morales-Ramos and Rojas 2013), suggesting that increased larval densities in the trays may have been the underlying cause for the problems occurring in the larval trays during 2012.

Larvae were fed only wheat bran during the period between January 2007 and February 2013. In March 2013, 5% dry potato squares were introduced to the diet of the growing larvae after nutritional studies showed a great increase in growth by addition of potato in the diet with minimal increase in cost (Morales-Ramos et al. 2013). Morales-Ramos and Rojas (2015) determined that increase in larval density resulted in decrease of food conversion efficiency and larval growth rate. In November 2015, a method to estimate larval density based on frass production, as described in the “larval Collection and Development” subsection, was implemented. Larval densities per tray were limited to a minimum of 10,000 and a maximum of 17,000. In August 2016, larval densities were lowered to a minimum of 7,000 and a maximum of 14,000 per tray. The time between eclosion and pupation decreased from a range of 9–12 mo to a range of 4–7 mo after the larval density control was established.

Separation of pupae from larvae originally was done using a standard No. 6 (3.35 mm) sift, but by the end of 2014 this sift size was no longer effective separating most larvae from pupae due to the larger sizes of larvae being produced. Starting in 2015 pupae separation was done using a sifter with larger openings (standard No.5, 4 mm) as described in the “Colony Reproduction” subsection.

**Selection for Larger Size**

Selection for larger size took place at the pupal stage at the time of selecting individuals for colony reproduction. The method of pupal selection for reproduction consisted of three steps: 1) damage and deformed pupae were removed; 2) the smallest pupae were removed; and 3) the largest pupae were selected. Selection of large pupae was done purely by visual inspection and no weighing was done during the selection process. Selection started with the largest pupae and continued with the next smaller sizes until a group of 350 pupae were collected. These 350 pupae were used to obtain between 250 and 300 adults for a single reproductive tray as described in the “Colony Reproduction” subsection. Pupal selection for larger size started in March 2010 and has continued uninterrupted until present time. The practice of recording daily numbers of pupae and their collective weight started on the same date. During this period of selection only one researcher has been responsible for selecting the pupae.

**Data Analysis**

Data from the colony log consisting of daily counts of pupae collected and their total weight in grams was analyzed in three ways for changes in body size. Average pupal weight was calculated by dividing the collective pupal weight by the number of pupae collected per
day. A linear regression model was used to determine if pupal weight was significantly impacted by time, which was represented in days starting at 0 in 25 March 2010 and continuing to 20 July 2018 at day 3,039. The data was also tested for quadratic effects on pupal weight. In addition to analyzing the whole raw data consisting of 3,040 observations with regression, means were calculated at 10 and 30 d intervals creating data sets of 303 and 101 data points, respectively. Means of 10 and 30 d intervals were analyzed using regression in the same way as it was done for the raw data to obtain models with lower variability. Four data points were eliminated from the raw data set after being identified as outliers using Cook’s D influence statistic (Freund et al. 2003).

Data was grouped in 265 data point groups using a nominal variable named ‘year’. Data of average pupal weight was compared among years using a mixed effects general linear model (GLM). The model consisted of the nominal variable ‘Year’ and the numerical variable ‘Number of Pupae.’ The numerical variable was added to calculate the daily average pupal weights. The means of pupal weight from each year were compared using the least square (LS) means Tukey–Kramer HSD test of JMP version 11 (SAS Institute 2007).

Preliminary Comparison of Ancestral and Selected Strains
An independent analysis was carried out at the facilities of a commercial insect farm (Betta Hatch, Seattle, WA), comparing the selected (Stoneville) strain with the ancestral colony originated from Southeastern Insectaries, Perry, GA (SEI). *Tenebrio molitor* larvae were purchased from Southeastern Insectaries in January 2017 to start the ancestral colony. The Stoneville colony was initiated from a shipment of last instars from Stoneville, MS in February 2017. Upon receiving the colony, adults completing development were placed in trays (25.4 × 50.8 × 5.7 cm) fed with wheat flour and carrots. Eggs obtained from these adults (F0) were sifted from the flour, transferred to new trays, and provided with organic wheat bran for development. Adult evaluations were done on the second generation (F1) after the larvae became adult beetles, to remove environmental effects from the colony sources. Both progeny of F0 and F1 adults were evaluated for larval traits. Food conversion, biomass production, and fecundity of the two strains were compared at room conditions of 27–29°C, 55–80% relative humidity, and no lights.

Food Utilization
Eight groups of 5–9-wk-old F1 larvae weighing 2 g (estimated to be 160 individuals) from each strain (total 16 groups) were placed in trays (25.4 × 50.8 × 5.7 cm) and provided with 61.5 g of wheat bran. Groups were maintained at the conditions described at the beginning of this section for up to 120 d. After 44 d, cups were monitored at variable intervals of between 7 and 10 d. Larvae were separated from the food and weighed. Food was separated from frass by using a No. 35 sieve (500 μm) and remaining food and frass were weighed. Dry weight gained (DWG) was calculated by multiplying live weight gained (LWG) by the dry weight proportion of *T. molitor* larvae (0.38) as reported by Finke (2002). Food consumed (FC) was calculated by subtracting the weight of the remaining food from the weight of the food provided. The water content of the food was determined before the start of the experiment by the difference between stored and dried food samples. The dry weight of remaining food and frass was estimated by subtracting water content proportions determined from freeze-dried samples.

Data Analysis
The food utilization parameters were calculated as described by Waldbauer (1968). Food assimilated (FA) was calculated as FA = FC – frass. Efficiency of conversion of ingested food (ECI) was calculated as ECI = DWG × 100 / FC and efficiency of conversion of assimilated food (ECA) was calculated as ECA = DWG × 100 / FA (Waldbauer 1968). Data of food utilization parameters were analyzed using mixed effects GLM and means of the different strains were compared using the LS means contrast at α = 0.05 of JMP version 11 software (SAS Institute 2007).

Fecundity
Groups of between 45 and 54 adults of the same age were collected from F1 pupae as they completed development and placed in 18.4 × 16.2 × 5.7 cm (24 oz) deli containers. As many groups as possible were collected from both colonies from October 6 to 13 December 2017. In total, 20 and 21 adult groups were collected from the SEI and Stoneville strains, respectively. Each adult group was provided with 160 g of wheat flour as food and oviposition substrate and maintained at the environmental conditions described above. Cups with adult beetles were monitored for oviposition every 6–14 d. Eggs were separated from the flour by sifting and weighed. The weight of the eggs, age of the adult group, and oviposition interval in days were recorded for each strain and group.

Data Analysis
Group pivotal age at each ovipositional period was calculated as (age at beginning of oviposition period) – (oviposition period/2). The number of eggs in each sample was estimated by dividing the total eggs weight by the mean individual weight of an egg as calculated from a sample of 173 and 210 eggs (0.5717 mg and 0.5843 mg individual weight) for SEI and Stoneville strains, respectively. Data consisting of estimated number of eggs per adult group per oviposition period was analyzed using mixed effects GLM. The model included one nominal variable (strain) and three numerical variables: number of adults, oviposition period in days, and pivotal age at ovipositional period. Differences in number of eggs per group and period was compared between strains by using LS means contrast of JMP version 11 software (SAS Institute 2007).

Larval Growth and Mortality
Eggs obtained from the fecundity experiment (F2) were used to estimate overall larval mortality and growth. Groups of variable number of eggs were placed in 13.7 × 11.4 × 6.4 cm (12 oz) cups. Egg groups of each of the two strains were weighed and their weight was recorded. In total, 50 egg groups were selected for each of the two strains making a total of 100 cups with eggs. Cups were provided with food consisting of wheat bran and maintained at the conditions described at the beginning of this section for up to 120 d. After 44 d, cups were monitored at variable intervals of between 7 and 10 d. Larvae were separated from the food and weighed, and their age and weight were recorded. A sample of 20 larvae was weighed from each cup to determine the mean larval weight in each cup. If pupae or adults were found, they were weighed and their weight was recorded in a cumulative weight associated with each cup and strain.

Data Analysis
The total biomass in each cup and age consisted of larval weight plus the accumulated weight of pupae and adults. Eggs and larvae were not counted and their numbers were estimated by weight using
the total weights divided by mean individual weights. The data was divided into four age classes comprising 20 d spans: 1) age 44–59; 2) age 60–70; 3) age 80–99; and 4) age 100–120. Larval survival in each cup at age 'I' was estimated by dividing estimated number of larvae at age 'I' by the initial estimated number of eggs.

Data of total biomass produced at every age was analyzed as a whole and by age classes using mixed effects GLM. The model included the independent variable strain, age, and number of eggs to evaluate their effect on the dependent variable biomass. Differences in biomass produced between the strains were compared using the LS means contrast of JMP version 11. Overall survival and survival at each of the four age classes were analyzed in similar way, but the model only included two independent variables, strain and age. Survival proportions 'P' were converted to ArcSin(SqRoot(P)) to eliminate the bias from the binomial distribution (Zar 1999).

Results
Pupal Size Increase
Overall, pupal weight increased from an average of 106.6 ± 0.43 (mean ± SEM) mg per individual in the first year to 177.2 ± 0.86 mg

![Graph showing pupal weight increase](image)

**Fig. 1.** Daily averages of pupal weight of *T. molitor* selected for larger size over a period of 8 yr. Circles represent means, the solid and dashed lines represent the linear and the quadratic regression models, respectively.

**Table 1.** Parameter estimates of linear and quadratic models using raw data (daily averages) and 10-d and 30-d means of pupal weight versus time

| Data source/parameter | Estimate   | Standard error | T ratio | Probability > |T| |
|-----------------------|------------|----------------|---------|---------------|---------------|
| **Linear model (Y = a + bX)** |             |                |         |               |               |
| Raw data (*R*² = 0.796; *F* = 11,751; df 1, 3,005; *P* < 0.0001) |             |                |         |               |               |
| Intercept             | 101.13733  | 0.392624       | 257.59  | <0.0001       |               |
| Slope                 | 0.0242009  | 0.000223       | 108.4   | <0.0001       |               |
| 10-d means (*R*² = 0.838; *F* = 1,558; df 1, 301; *P* < 0.0001) |             |                |         |               |               |
| Intercept             | 101.2278   | 1.073647       | 94.11   | <0.0001       |               |
| Slope                 | 0.2423529  | 0.006139       | 39.48   | <0.0001       |               |
| 30-d means (*R*² = 0.855; *F* = 589.5; df 1, 100; *P* < 0.0001) |             |                |         |               |               |
| Intercept             | 101.51365  | 1.746939       | 58.11   | <0.0001       |               |
| Slope                 | 0.7255862  | 0.029884       | 24.28   | <0.0001       |               |
| **Quadratic model (Y = a + bX + cX²)** |             |                |         |               |               |
| Raw data (*R*² = 0.811; *F* = 6,438; df 2, 3004; *P* < 0.0001) |             |                |         |               |               |
| Intercept             | 97.965658  | 0.433107       | 226.19  | <0.0001       |               |
| b                     | 0.0241913  | 0.000215       | 112.37  | <0.0001       |               |
| c                     | 0.000004146| 0.00000027     | 15.08   | <0.0001       |               |
| 10-d means (*R*² = 0.853; *F* = 871.8; df 2, 300; *P* < 0.0001) |             |                |         |               |               |
| Intercept             | 98.019496  | 1.177711       | 83.23   | <0.0001       |               |
| b                     | 0.2423816  | 0.005856       | 41.39   | <0.0001       |               |
| c                     | 0.0004148  | 0.0000747      | 5.55    | <0.0001       |               |
| 30-d means (*R*² = 0.869; *F* = 328.4; df 2, 99; *P* < 0.0001) |             |                |         |               |               |
| Intercept             | 98.450896  | 1.914942       | 51.41   | <0.0001       |               |
| b                     | 0.7255862  | 0.028543       | 25.42   | <0.0001       |               |
| c                     | 0.0035329  | 0.001084       | 3.26    | 0.0015        |               |
per individual over the course of 8-yr selection period. Regression analysis results from the raw daily data of pupal weights showed significant effect of time on pupal weight ($R^2 = 0.796; F = 11,751; df = 1, 3005, P < 0.0001$). Parameter estimate showed a positive value of the slope ($b = 0.0242$), indicating that pupal weight increased over time. The data fit the quadratic model slightly better ($R^2 = 0.811; F = 6438; df = 2, 3004; P < 0.0001$), with a positive value of the quadratic parameter ($c = 0.000004$) suggesting a slight acceleration in the rate of pupal size increase over time (Fig. 1). Models using the 10 and 30-d means produced similar results, but the parameter values were higher (Table 1).

Pupal weight seemed to change in an oscillating manner over time consisting of periods of rapid increase following by periods of decline. This pattern was more evident when data consisting of 30-d means of pupal weight was plotted over time (Fig. 2).

Comparison of 365-d means of pupal weight using GLM showed significant differences among LS means ($F = 1488; df = 9, 2997; P < 0.001$). All LS means of pupal weight of years 0–8 indicated significant yearly increase, except between years 3 and 4 where there was not a significant increase in pupal weight. The ANOVA results were not different ($F = 1622; df = 8, 2998; P < 0.001$) (Table 2), but the GLM results eliminated significant bias originating from the varying number of pupae from daily counts (Parameter $= -0.0094; T$ ratio $= -8.92; P < 0.0001$), which were used to calculate daily averages of pupal weight. The negative value of the parameter indicated that smaller groups of pupae tended to yield slightly higher pupal weight averages and vice versa.

**Preliminary Comparison of Ancestral and Selected Strains**

Significant differences were found between the ancestral (Southeastern Insectaries) and the selected (Stoneville) strains. In the food utilization experiment, differences were observed in biomass gained ($F = 11.14; df = 1, 13; P = 0.0053$) and ECI ($F = 7.8; df = 1, 13; P = 0.0152$), and in both measures the Stoneville strain was superior to the SEI strain (Table 3). There was no significant difference in food assimilation between the two strains. Differences were also significant in fecundity between the two strains ($F = 48.19; df = 3, 320; P < 0.0001$), where the Stoneville strain produced more eggs than the SEI strain (Table 3). Larval survival differed significantly between the strains ($F = 61.62; df = 1, 327; P < 0.0001$), where the SEI strain outperformed the Stoneville strain. Differences in survival between the two strains were significant for age classes 1 and 2 (Table 4). Pupation started at the beginning of age classes 3 and survival analyses of age classes 3 and 4 were discarded because of bias generated by the removal of pupae. Live biomass gain differ significantly between the strains ($F = 5.55; df = 1, 326; P = 0.019$) when the data was analyzed as a whole, but biomass gain during age classes 1–3 did not differ significantly between strains (Table 4).

**Discussion**

Average pupal weight of *T. molitor* showed a significant increase after applying continuous selection for larger size pupae during a period of 8 yr. Colony protocols were improved and refined during that period of time and some of those improvements, especially larval density reduction in trays, may have some influence on pupal size changes. Weaver and McFarlane (1990) reported increasing size in *T. molitor* pupae as larval densities decreased. However, the continuous increase in size (except between years 3 and 4) and the apparent acceleration in the rate of size increase point to selection as the main factor causing pupal size increase.

Some colony crises occurred over the 8-yr period. For instance, during 2012, pupal production dropped to critically low levels due to several factors including disease, overcrowding, and an undetected humidifier malfunction. Overcrowding of larvae occurred when reduction of adult density increased progeny survival (Morales-Ramos et al. 2012). This resulted in more larvae going into new trays, a shift that could not be measured due to the lack of methods to estimate early instar densities. This overcrowding of larvae subsequently made larvae susceptible to fungal diseases. During the crisis, pupal selection was completely relaxed because all of the pupae were used to maintain colony reproduction. This may have been responsible, at least in part, for the lack of increase in pupal size during years 3 and 4, which correspond to 2013–2014. It should be noted that in 2012 the generation time of *T. molitor* in the Stoneville colony was longer than 1 yr with the suboptimal conditions of larval and adult densities in the colony. Under current conditions, the generation time has been reduced to 7–8 mo.

Pupal size increase rates show an undulating pattern over time that seems to indicate oscillation in pupal size over time. These oscillation waves in pupal size may represent cohorts of larvae where smaller sizes develop faster and pupate earlier than larger sizes, which pupate at a later age. This hypothesis needs to be tested in a future study with more detail.
The independent comparison of ancestral versus selected strains showed significant differences in larval growth rate, efficiency of food conversion, fecundity, and larval survival between the two strains. The selected strain (Stoneville) appears to have a better food conversion efficiency, a higher larval growth rate, and higher fecundity than the ancestral strain (SEI). These results are consistent with observations made by Leclercq (1963) of increased growth, development rate and fecundity in T. moli-

or after selecting for larger pupal size over 7 yr. However, the selected strain also displays lower larval survival than the ancestral strain. In general, the advantages in reproduction and growth of the selected strain can compensate for the lower larval survival, but more detailed comparisons are required to draw conclusions about their suitability as a production strain. Also, it may be possible that after periods of selection followed by periods of relaxed selection, the larval survival may improve in the Stoneville strain. Whether this practice may cause decline in pupal size remain to be tested.

In conclusion, selecting for larger size in T. molitor not only can result in strains of larger size, but also can result in strains with higher biomass productivity. However, selecting for larger size may also result in the increase of some detrimental characters, such as lower larval survival. Future studies will focus on investigating the relationship between pupal size and development time and between pupal size and progeny survival and the possibilities to further select for higher larval survival in a large-sized strain.

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References Cited
Aguilar-Miranda, E. D., M. G. López, C. Escamilla-Santana, and A. P. Barba de la Rosa. 2002. Characteristics of maize flour tortilla supplemented with ground Tenebrio molitor larva. J. Agric. Food Chem. 50: 192–195.

Barker, D., M. P. Fitzpatrick, and E. S. Dierenfeld. 1998. Nutrient composition of selected whole invertebrates. Zoo. Biol. 17: 123–134.

Barroso, F. G., C. de Haro, M.-J. Sánchez-Muros, E. Venegas, A. Martínez-Sánchez, and C. Pérez-Bañón. 2015. The potential of various insect species for use as food for aquaculture. Aquaculture 422-423: 193–201.

Belfort, M., F. Gai, C. Lussiana, M. Renna, V. Malfatto, L. Rotolo, M. De Marco, S. Dabbou, A. Schiavone, I. Zoccarato, et al. 2015. Tenebrio molitor meal in diets for the European sea bass (Dicentrarchus labrax L.): juveniles: growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Tech. 220: 34–45.

Halloran, A., N. Roos, J. Eilenberg, and A. Cerutti. 2016. Life cycle assessment of edible insects for food and protein: a review. Agron. Sustain. Dev. 36: 57.

Halloran, A., H. H. Hansen, L. S. Jensen, and S. Bruun. 2018. Comparing environmental impacts from insects for feed and food as an alternative to animal production, pp. 163–180. In A. Halloran, R. Flore, P. Vantomme, and N. Ross (eds.). Edible insects in sustainable food systems. Springer, Cham, Switzerland.

Heckmann, L.-H., J. L. Andersen, N. Gianottom, M. Calis, C. H. Fischer, and H. Calis. 2018. Sustainable mealworm production for feed and food, pp. 321–328. In A. Halloran, R. Flore, P. Vantomme, and N. Ross (eds.). Edible insects in sustainable food systems. Springer, Cham, Switzerland.

van Huis, A. 2017. New sources of animal proteins: edible insects, pp. 443–461. In P. P. Purslow (ed.), New aspects of meat quality: from genes to ethics. Elsevier, Duxford, United Kingdom.

van Huis, A., and F. V. Dunkel. 2016. Edible insects: a neglected and promising food source, pp. 341–355. In S. Badathur, J. P. D. Wanasandra, and L. Scanlin (eds.), Sustainable protein sources. Academic Press, London, United Kingdom.

van Huis, A., J. Van Itterbeeck, H. Kundra, E. Mertens, A. Halloran, G. Muir, and P. Vantomme. 2013. Edible insects, future prospects for food and feed security, pp. 187. FAO, ONU, Rome, Italy.

van Huis, A., M. Dicke, and J. J. A. vanloon. 2015. Insects to feed the world. J. Ins. Food Feed. 1: 3–5.

Leclercq, J. 1963. Artificial selection for weight and its consequences in Tenebrio molitor L. Nature 198: 106–107.

Makkar, H. P. S., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Tech. 197: 1–33.

Manzano-Aguiglieri, F., M. J. Sánchez-Muros, F. G. Barroso, A. Martínez-Sánchez, S. Rojo, and C. Pérez-Bañón. 2012. Insects for biodiesel production. Renew. Sust. Energ. Rev. 16: 3744–3753.

Martin, R. D., J. P. W. Rivers, and U. W. Cowgill. 1976. Culturing mealworms as food for animals in captivity. Int. Zoo Yearb. 16: 63–70.

Moraless-Ramos, J. A., and M. G. Rojas. 2015. New sources of animal proteins: edible insects, pp. 443–461. In P. P. Purslow (ed.), New aspects of meat quality: from genes to ethics. Elsevier, Duxford, United Kingdom.

Morales-Ramos, J. A., M. G. Rojas, J. A. Morales-Ramos, S. Kay, D. I. Shapiro-Ilan, and W. L. Tedders. 2012. Impact of adult weight, density, and age on reproduction of Tenebrio molitor (Coleoptera: Tenebrionidae). J. Econ. Entomol. 105: 2259–2267.

Morales-Ramos, J. A., M. G. Rojas, D. I. Shapiro-Ilan, and W. L. Tedders. 2013. Use of nutriment self-selection as a diet refining tool in Tenebrio molitor (Coleoptera: Tenebrionidae). J. Insect Sci. 13: 206–221.

Ng, W.-K., F.-L. Liew, L.-P. Ang, and K.-W. Wong. 2001. Potential of mealworm (Tenebrio molitor) as an alternative protein source in practical diets for African catfish, Clarias gariepinus. Aquacult. Res. 32: 272–280.

Oonincx, D. G. A. B., and I. J. M. de Boer. 2012. Environmental impact of insects for biodiesel production. Renew. Sust. Energ. Rev. 16: 3744–3753.

Oonincx, D. G. A. B., and I. J. M. de Boer. 2012. Environmental impact of production of mealworms as a protein source for humans—a life cycle assessment. PLoS One 7: e13145.

Oonincx, D. G., J. van Itterbeeck, M. J. Heetkamp, H. van den Brand, J. J. van Loon, and A. van Huis. 2010. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PLoS One. 5: e11445.

Pal, P., and S. Roy. 2014. Edible insects: future of human food—a review. Int. Lett. Nat. Sci. 21: 1–11.

Payne, C. L., P. Scarborough, M. Rayner, and K. Nonaka. 2016. Are edible insects more or less ‘healthy’ than commonly consumed meats? A comparison using two nutrient profiling models developed to combat over- and undernutrition. Eur. J. Clin. Nutr. 70: 285–291.

Ramos-Elorduy, J. 1997. Insects: a sustainable source of food? Ecol. Food Nut. 36: 247–276.
Ramos-Elorduy, J. 2009. Anthropo-entomophagy: cultures, evolution and sustainability. Entomol. Res. 39: 271–288.

Ramos-Elorduy, J., E. A. González, A. R. Hernández, and J. M. Pino. 2002. Use of Tenebrio molitor (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. J. Econ. Entomol. 95: 214–220.

Sánchez-Muros, M. J., F. G. Barroso, and C. de Haro. 2016. Brief summary of insect usage as an industrial animal feed/ingredient, pp. 273–309. In A. T. Dossey, J. A. Morales-Ramos, and M. G. Rojas (eds.), Insects as sustainable food ingredients: production, processing and food applications. Academic Press, San Diego, CA.

SAS Institute. 2007. JMP release 7: statistics and graphics guide. SAS Institute, Inc., Cary, NC.

Shapiro-Ilan, D. I., J. A. Morales-Ramos, and M. G. Rojas. 2016. In vivo production of entomopathogenic nematodes, pp. 137–158. In T. R. Glare and M. E. Moran-Diaz (eds.), Microbial-based biopesticides: methods and protocols, methods in molecular biology, vol. 1477. Springer Science & Business media, New York, NY.

Shockley, M., and A. T. Dossey. 2014. Insects for human consumption, pp. 617–652. In J. A. Morales-Ramos, D. I. Shapiro-Ilan, and M. G. Rojas (eds.), Mass production of beneficial organisms, invertebrates and entomopathogens. Academic Press, Waltham, MA.

Shockley, M., J. Lesnik, R. N. Allen, and A. F. Muñoz. 2018. Edible insects and their uses in North America; past, present and future, pp. 55–79. In A. Halloran, R. Flore, P. Vantomme, and N. Ross (eds.), Edible insects in sustainable food systems. Springer, Cham, Switzerland.

Smetana, S., M. Palanisamy, A. Mathys, and V. Heinz. 2016. Sustainability of insect use for feed and food: life cycle assessment perspective. J. Cleaner Prod. 137: 741–751.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Advan. Ins. Physiol. 5: 229–288.

Weaver, D. K., and J. E. McFarlane. 1990. The effect of larval density on growth and development of Tenebrio molitor. J. Ins. Physiol. 36: 531–536.

Zar, J. H. 1999. Biostatistical analysis, 4th ed, pp. 123. Prentice-Hall, Inc., Upper Saddle River, NJ.