1   **Effects of maternal age and stress on offspring quality in a viviparous fly**

2

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10 **Statement of authorship**

11 SE and LH designed the study with inputs from ST, JSL and MB. RL and LH carried out the
12 laboratory experiments. JSL carried out the analyses with input from SE, MB and ST and led
13 the writing of the manuscript. RL, LH, AB, MB, ST and SE contributed to manuscript
14 content and structure.

15 **Data accessibility statement**

16 The data and R scripts to reproduce the analyses will be available at

17 https://github.com/jenniesuz/tsetse_senescence.git.

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Abstract

Many organisms show signs of deterioration with age, both in terms of survival and reproduction. Theory suggests that variation in such senescence patterns can be driven by resource availability or reproductive history. Here, we test this theory by manipulating nutritional stress and age at first reproduction and measuring age-dependent reproductive output in tsetse (*Glossina morsitans morsitans*), a viviparous fly with high maternal allocation. Across all treatments, offspring wet weight followed a bell-shaped curve with maternal age. Nutritionally stressed females had higher probability of abortion, produced smaller offspring with lower starvation tolerance. Despite this, there was no strong evidence of differences between treatments in the pattern of abortion probability, offspring wet weight or offspring starvation tolerance with age. Therefore, although we found strong evidence of general reproductive senescence in tsetse, variation in the onset and rate of senescence was not explained by resource allocation trade-offs or the costs of reproduction.

Introduction

Across the natural world, it is commonly observed that as individuals get older, they are more likely to die and have lower reproductive output (Nussey *et al.* 2013; Hoekstra *et al.* 2019; Zajitschek *et al.* 2019). Broad patterns of actuarial and reproductive senescence have been well described for a wide range of taxa (Nussey *et al.* 2013; Hoekstra *et al.* 2019; Zajitschek *et al.* 2019), particularly birds and mammals (Gaillard & Lemaître 2020). There is also extensive variation in the onset and rate of ageing, both within and across populations (Holand *et al.* 2016; Rodríguez-Muñoz *et al.* 2019; Cayuela *et al.* 2020). The role of individual heterogeneity, and subsequent variation in the onset and rate of ageing among individuals, however, has been neglected (Gaillard & Lemaître 2020).

A central tenet of the evolution of senescence is that the strength of selection declines with age (Hamilton 1966), with consequences in terms of antagonistic pleiotropy (Williams 1957) or the accumulation of somatic damage (Kirkwood 1977). Life-history theory suggests both antagonistic pleiotropy and the accumulation of somatic damage can be explained by trade-offs between reproduction and other physiological processes (Kirkwood 1977; Boggs 2009; Baudisch & Vaupel 2012; Davison *et al.* 2014). A key assumption underlying this theory is that resources are limited and must be allocated either to reproduction or somatic maintenance (Partridge 1987; Boggs 2009). Reproductive senescence could also occur through physiological damage incurred directly from reproduction, or can be an adaptive strategy to prolong survival (McNamara *et al.* 2009).

Experimental manipulation of access to mates or food can yield insights on the factors driving variation in senescence, including costs of reproduction and allocation of resources. For such experiments, insects are useful organisms as they have relatively short generation times, can be reared in large numbers, and access to mates and resources can be easily manipulated. Experiments with Lepidoptera, for example, have shown that delayed mating reduces fecundity but extends longevity (Unnithan & Paye 1991; Jiménez-Pérez & Wang 2009). A large body of studies have also shown that females with access to fewer resources have lower overall reproductive output but longer lifespan (De Sousza Santos & Begon 1987; Ernsting & Isaaks 1991; Kaitala 1991; Chippindale *et al.* 1993; Tatar & Carey 1995; Curtis Creighton *et al.* 2009). These studies support the theory that senescence is, at least in part, caused by direct costs of reproduction through physiological damage, or arises as an indirect

97 consequence of resource availability. However, experimental manipulation of both age at

98 reproduction and nutrition is required to tease apart the contributions of reproductive history

99 and resource availability to senescence.

100

101 Here, we present such an experimental study, focussing on reproductive senescence in tsetse

102 (*Glossina morsitans morsitans*). Tsetse are vectors of human and animal trypanosomiasis in

103 Africa. They give birth to a single live larva weighing the same as the mother (Hargrove &

104 Muzari 2015; Haines *et al.* 2020), approximately every nine days. Immature stages receive

105 energy and nutrients from the mother only. Adults do not increase in size after emergence and

106 have a relatively long lifespan for their small size, living for weeks rather than days

107 (Hargrove *et al.* 2011).

108

109 Evidence for age-related changes in reproductive output in field and laboratory tsetse is

110 mixed (Jordan *et al.* 1969; Langley & Clutton-Brock 1998; McIntyre & Gooding 1998). Key

111 limitations to these studies are that flies were kept only under optimal laboratory conditions,

112 not tracked individually and frequently grouped across ages. Preliminary data from the tsetse

113 colony at the Liverpool School of Tropical Medicine (LSTM) provided evidence of

114 reproductive senescence (Supporting Information, S1 File), but these data did not allow for

115 teasing apart of within-individual from among-individual patterns (Monaghan *et al.* 2020). In

116 this study, we therefore used a novel method of housing tsetse females to track individual

117 mothers and their offspring from this colony.

118

119 Our first objective was to confirm reproductive senescence in tsetse, as shown by a decline in

120 the number and quality of offspring with maternal age. Our second objective was to

121 determine the effect of maternal nutrition and the physiological costs of reproduction on age-

122 dependent patterns of maternal allocation. To achieve this aim, we manipulated nutritional

123 stress by feeding adult females on a high- or low-quality diet and we varied potential costs of

124 reproduction by delaying the age at which females were mated. Our third objective was to

125 assess variation in reproductive senescence patterns among individuals, due to unmeasured

126 heterogeneity in factors including size, condition and mate quality.

127

128 We hypothesised that: i) females mated later would experience a delayed and/or slower

129 increase in the probability of abortion and decline in offspring quality, due to physiological

130 costs of reproduction, resource allocation trade-offs, or both; and either: ii) nutritionally

131 stressed mothers would have an earlier, and potentially steeper increase in probability of

132 abortion and decline in offspring quality, due to resource allocation-trade-offs; or iii)

133 nutritionally stressed mothers may senescence more slowly, due to lower physiological costs

134 of reproduction, if stressed females have lower reproductive output.

135

Methods

137 Tsetse from a colony of *G. m. morsitans* maintained at LSTM were used for the experiments.

138 The colony was established using pupae from Zimbabwe and has been maintained at LSTM

139 since 2004. The colony was kept at 26°C (± 2 °C), 68–76% relative humidity and a 12 h

140 photoperiod. Flies were fed on a Monday, Wednesday and Friday on defibrinated horse blood

141 (TSC Bioscience) which comprises 40–50% red blood cells.

142

Measures of offspring quality

144 In addition to spontaneous abortions, we recorded the wet weight of viable pupae and, for a

145 subset, we measured either fat, or whether they emerged and their starvation tolerance upon

146 emergence (Fig. 1). Wet weight of pupae correlates with pupal volume in field flies and does

147 not require destructive sampling (Hargrove 1999). We recorded emergence as we

148 hypothesised that offspring with more reserves would be more likely to emerge. We

149 measured starvation tolerance because, in the wild, newly emerged tsetse have a high

150 probability of mortality (Hargrove *et al.* 2011), likely due to failure to find a blood meal

151 before running out of energy reserves.

152

153 From preliminary data on the tsetse colony at LSTM (S1 File), we hypothesised that there

154 would be an increase in the probability of abortion with age. If resource-allocation trade-offs

155 exist we would also expect that the increase would be steeper for the nutritionally stressed

156 treatment and potentially shallower for the mating delay treatment, relative to the control.

157 However, for offspring wet weight and starvation tolerance we predicted that there would be

158 an initial increase, reflecting the requirement for newly emerged flies to also divert energy

159 and nutrients to the development of thoracic muscles (Anderson & Finlayson 1973), followed

160 by a decline due to senescence. Lastly, we hypothesised that the effect of maternal age on

161 starvation tolerance would be determined largely by energy reserves, as indirectly measured

162 by offspring wet weight. Heavier offspring would have more reserves and therefore would be

163 more likely to emerge and have higher starvation tolerance. Maternal age could also influence

164 offspring starvation tolerance, independent of effects on wet weight, through the quality of

165 the resources provided.

166

Selection and mating of female tsetse for the experiment

168 The *G. m. morsitans* colony at LSTM is maintained in 12 trays corresponding to each week

169 of life. Females in the colony older than this are killed due to a decline in reproductive

170 output. To select females for the experiment, we collected equal numbers of pupae from each

171 tray over a one-week period for emergence (S2 File). At the time of mating (upon emergence

172 or after a delay), females were mated at a ratio of 2:1 (female:male) with one-week old males

173 for 48 hours. Males were then removed, and females were selected for the experimental

174 treatments.

175

Treatments

177 We set up control, nutritional stress and mating delay treatments, each consisting of 96 adult

178 female *G. m. morsitans* (Fig. 1, S2 File). Because reducing the amount of haemoglobin in a

179 bloodmeal results in lower pupal wet weights (Kabayo & Langley 1985), we chose to dilute

180 red blood cells with serum to produce a low-quality diet for the nutritional stress group. Trials

181 testing different ratios of red blood cells to serum showed that flies fed on c. 10% red blood

182 cells produced lighter pupae but had similar survival over a 50-day period, compared to flies

183 fed on c. 45% red blood cells (S3 File). We thus ascribed the 10% red blood cell treatment as

184 the 'nutritional stress' treatment and 45% red blood cells as 'control'. For the mating delay

185 treatment, virgin females were kept in communal cages for three weeks post-emergence.

186 Virgin females continue to ovulate, but mature eggs eventually disintegrate (Ejezie & Davey

187 1977). Once mated, they were separated into individual cages. The experiment was then run

188 until mothers were 100 days old. (Hargrove *et al.* 2011). In addition, unpublished data from

189 the LSTM colony indicated that by 100 days the probability of abortion had increased from c.

190 0 to 0.25 and the size of pupae produced had declined to similar sizes seen from first-time

191 mothers (S1 File).

192

Housing of individual adult females and feeding regime

194 Flies were housed in individual cages, which were placed on pupal collection trays, made

195 from acrylic extrude (described in S2 File), to allow individual tracking of female

196 reproductive output and survival. Cameras were fixed below the cages to record weekend

197 activity and to identify exact larviposition dates. Females were monitored daily between

198 Monday and Friday and if death occurred, we recorded the date of death. To determine the

199 exact date of death for females found dead on a Monday morning, we reviewed video

200 recordings from Friday evening to Monday morning (S2 File).

201

202 Experimental flies were fed following the colony schedule (see above). To feed individual

203 females, we placed cages on a shallow grid (S2 File). The grid ensured each female was

204 provided with the same amount of blood and had equal opportunity for feeding, with c. 100

205 μl of blood provided per female. We heated the tray to 37°C and covered the blood with a

206 silicon membrane, then left flies to feed for 45 minutes, which was sufficient time for most

207 flies to feed.

208

209 **Offspring measurements**

210 Time of larviposition was recorded, including whether or not larvae produced were viable

211 (Baldry *et al.* 1992). Production of non-viable offspring was considered a spontaneous

212 abortion ('abortion' hereafter). It was not possible to record egg abortions as they were not

213 visible due to their small size. For pupae collected on a Monday morning, we consulted the

214 video recordings to determine the exact day of larviposition (S2 File). For aborted larvae,

215 however, we could not determine the date for those aborted over the weekend, because early

216 larval stages were also not visible on the camera and were therefore recorded as occurring on

217 the Friday. For viable pupae, we measured the wet weight, to 0.1 mg. aining 30% were

218 destructively sampled for fat analysis (S2 File) in order to quantify how wet weight correlates

219 with fat reserves.

220

221 Pupae assigned for emergence studies were placed singly into 50 ml Falcon tubes with a 3

222 mm hole drilled in the centre of the screw cap to allow air flow. Pupae were observed daily

223 for date of emergence on working days and again video recordings were used to determine

224 the date of emergence on weekends. Pupae were observed for a maximum of 50 days. Any

225 pupae that had not emerged by 50 days, were recorded as a failed emergence (Hargrove

226 2004). Each emerging fly remained in the tube until it died of starvation. Sex of emerging

227 flies and date of death were recorded.

228

229 **Statistical analyses**

230 For each analysis described below, models were compared using Akaike's information

231 criterion (AIC). If the ratio of the sample size to the number of model parameters was <40,

232   we used AIC corrected for small sample size (AICc) (Burnham & Anderson 2002). Figures

233   were produced using the model with the lowest AIC. For model comparison, the difference in

234   AIC between each model and the lowest AIC model (Δ_i) and Akaike weights (ω_i) were

235   calculated. Akaike weights sum to one and provide a relative indication for the weight of

236   evidence for any one model as the best approximating model (Burnham & Anderson 2002).

237   We analysed the data using R version 3.6 (R Core Team 2014). The data and R scripts to

238   produce the results can be accessed at (https://github.com/jenniesuz/tsetse_senescence.git).

239

240   For the probability of abortion, offspring wet weight, and starvation tolerance, we carried out

241   statistical analyses for each treatment separately using linear and generalised linear mixed

242   effects models implemented with the 'lme4' and 'nlme' R packages (Bates *et al.* 2015;

243   Pinheiro *et al.* 2018). For each analysis described below maternal age in days was

244   incorporated as a continuous variable. All models with and without random effects were

245   fitted and simultaneously compared using maximum likelihood estimation, assuming that the

246   bias in the variance components would be relatively small ($n/(n-p)$: 1.009 for the probability

247   of abortion; 1.049 for offspring wet weight; and 1.095 for offspring survival, largest values

248   across treatments reported). Models with and without a random intercept were compared to

249   assess evidence for variation among individual mothers in the probability of abortion,

250   offspring wet weight and offspring starvation tolerance. We also compared models with and

251   without a random slope for maternal age, to assess evidence for variation in senescence

252   patterns among individual mothers.

253

254   The effect of maternal age on probability of abortion, offspring wet weight and offspring

255   starvation tolerance were compared between treatments using fitted coefficients and 95%

256   confidence intervals from the model with the lowest AIC. We took this approach to analysis

257   rather than including treatment as a factor in models as it is simpler and does not require the

258   assumption of equal variance between treatments.

259

260   Logistic regression was used to quantify the effect of maternal age on the probability of

261   abortion for each treatment, assuming a linear relationship between maternal age and the log

262   odds of abortion. For the effect of maternal age on offspring wet weight and starvation

263   tolerance, models including maternal age as a cubic, quadratic, logistic or linear effect were

264   compared. For wet weight, we also compared the most parsimonious model with a model fit

265   using generalised additive modelling (GAM). Cubic regression splines were fitted for each

266   treatment, with maternal age as the explanatory variable and accounting for multiple

267   offspring from individual mothers, using the mgcv R package (Wood 2017). Generalised

268   additive model fits with knots – locations where the slope changes – ranging from 3 to 10

269   were compared using AICc. The correlation between offspring wet weight and fat was

270   summarised using Pearson's correlation coefficient.

271

272   For offspring starvation tolerance, there was no censoring and the data were approximately

273   normally distributed (S4 Fig.). The relationship between offspring wet weight, sex, maternal

274   age and number of days to starvation was therefore modelled using linear mixed effects

275   models for each treatment. Maternal age was included in models as described for offspring

276   wet weight. In addition to maternal age and wet weight, we also included offspring sex as

277   females are larger than males on emergence (Hargrove *et al.* 2019).

278

279   We repeated the above analyses for the nutritional stress treatment, excluding females that

280   had died, to ensure our results were not affected by the possibility that females who died

281   allocated more to their offspring. For the other two treatments this was not done as >90% of

282   mothers were still alive by the end of the experiment. Lastly, an analysis of maternal survival

283   is provided in S5 File.

284

285   **Results**

286

287   **Abortions**

288   The probability of abortion increased with age across all treatments (models without age:

289   control $\omega = 0.000$; mating delay $\omega = 0.001$; and nutritional stress $\omega = 0.00$) (Tables S6 1-3).

290   The increase in the odds of abortion with age were similar across treatments (from the model

291   with lowest AIC: control 1.072, C.I. 1.054 – 1.094; mating delay 1.053, CI 1.025 – 1.086;

292   and nutritional stress 1.047, CI 1.034 – 1.061) (Fig. 2, S6 4-6). Females in the nutritional

293   stress treatment had higher probability of abortion at any age compared with the other two

294   treatments (Fig. 2).

295

296   For all treatments, including a random intercept and slope in models resulted in a singular fit

297   indicating that models were overfitted. We did not therefore assess evidence for individual

298   variation in the effect of maternal age on probability of abortion. There was, however, strong

299 evidence for variation among individual mothers in the probability of abortion for the

300 nutritional stress treatment (model with random intercept $\omega = 0.985$; model without $\omega =$

301 0.015). This was not the case for the control (model with random intercept $\omega = 0.289$; model

302 without $\omega = 0.711$) or mating delay treatments (model with random intercept $\omega = 0.368$;

303 model without $\omega = 0.631$) (Tables S6 1–3). Plots of the raw data for this analysis, and

304 analyses described below, can be viewed in S7 File.

305

306 **Offspring wet weight and fat**

307 Across all treatments, wet weight of pupae increased and then declined with maternal age

308 until the end of the experiment (Fig. 3). Model selection results provided strong evidence for

309 a quadratic effect of maternal age on offspring wet weight (control – quadratic effect

310 including random intercept and slope $\omega = 0.719$; mating delay – quadratic effect with random

311 intercept and slope $\omega = 0.562$, without random slope $\omega = 0.272$; and nutritional stress –

312 quadratic effect with random intercept and slope $\omega = 0.866$) (Tables S6 7-9). There was no

313 support for a log, or a linear effect of maternal age for any of the treatments ($\omega = 0.000$). In

314 addition, across treatments, the predicted effect of mother age at the population level,

315 assuming a quadratic fit, was within the standard errors of predictions from GAM fits to the

316 data (S8 File).

317

318 The fitted quadratic curves and coefficients were similar between treatments (from the model

319 with lowest AIC, which included a random intercept and slope, coefficient for maternal age:

320 control 0.520 (0.466 – 0.574); mating delay 0.564 (0.406 – 0.723); and nutritional stress

321 0.502 (0.420 – 0.584), coefficient for maternal age squared: control -0.0040 (-0.0045 – -

322 0.0035); mating delay -0.0042 (-0.0054 – -0.0030); and nutritional stress -0.0040 (-0.0048 – -

323 0.0032)) (Tables S6 10 - 12).

324

325 For all three treatments, there was strong evidence for individual variation among mothers in

326 the wet weight of their pupae (for all treatments, all models without random effects $\omega =$

327 0.000). This variation was highest in the mating delay treatment with a random intercept

328 variance from the most parsimonious model of 11.820, compared with 7.307 for the control

329 and 2.379 for the nutritional stress treatment (Tables S6 13 - 15).

330

331 There was evidence for variation among mothers in the effect of maternal age on wet weight

332 for the control and nutritional stress treatments (quadratic fit with random intercept and slope:

333   control $\omega = 0.719$; and nutritional stress $\omega = 0.866$). There was less support for this in the

334   mating delay treatment (quadratic fit with random intercept and slope $\omega = 0.562$, next lowest

335   AIC model - quadratic fit with random intercept only $\omega = 0.272$).

336

337   Offspring fat increased linearly with offspring wet weight (Pearson's correlation coefficient

338   0.554) (File S9), thus for brevity we focus on wet weight as our trait of interest, but the

339   patterns are qualitatively similar if offspring fat is considered instead.

340

341   **Offspring emergence and starvation tolerance**

342   During the 50-day pupal observation period, 96% of 355 pupae from the control, 93% of 187

343   pupae from the mating delay, and 91% of 232 pupae from the nutritional stress group

344   emerged successfully. All emerged offspring subjected to starvation were dead by 15 days

345   post emergence. Although the wet weight of pupae was similar between females and males

346   (Fig. 4a), across all three treatments there was strong evidence that female offspring survived

347   on average longer than males, but not by more than a day (Tables S6 19 – 21), with $\omega \leq 0.01$

348   for models not including sex (Tables S6 16 - 18, Fig. 4b).

349

350   There was strong evidence for an additional effect of maternal age on offspring starvation

351   tolerance, in addition to offspring wet weight, for the nutritional stress treatment (models

352   without age $\omega = 0.000$). Offspring from young mothers in the nutritional stress treatment

353   were particularly vulnerable to starvation (Fig. 4b), with evidence for a cubic effect of

354   maternal age in this treatment (cubic effect including random intercept $\omega = 0.705$). While

355   there was also evidence for maternal age effects on offspring starvation tolerance in the other

356   two treatments, there was not sufficient information in the data to quantify the relationship

357   between maternal age and offspring starvation tolerance for the control or mating delay

358   treatments (lowest AIC model: control – quadratic effect and no random effects $\omega = 0.298$;

359   mating delay – quadratic effect with no random effects $\omega = 0.212$) (Tables S6 16-17).

360

361   For the mating delay treatment, singular fits were obtained for models assuming a quadratic

362   or cubic effect of mother age and including a random intercept and slope, therefore there was

363   not sufficient data to assess for this treatment whether differences among individual mothers

364   carried through to emerged offspring starvation tolerance. There was also insufficient

365   information in the data to provide evidence for among-mother variation in the control

366   treatment, with the difference in AIC weights between models with and without random

367 effects <0.15 (Tables S6 16 - 18). However, there was evidence for an effect of individual

368 mother on starvation tolerance in the nutritional stress treatment (all models without random

369 effects $\omega \leq 0.04$) (Tables S6 16 - 18), but not for variation among individual mothers in the

370 effect of maternal age on offspring starvation tolerance ($\omega \leq 0.005$).

371

372 The results of the above analyses for the probability of abortion, offspring wet weight and

373 offspring starvation tolerance were unaffected by removing from the analysis mothers in the

374 nutritional stress treatment that had died before the end of the experiment. The analyses can

375 be found in S10 File and compared with results in S6 File.

376

377 **Discussion**

378 While there is substantial evidence of actuarial senescence in insects and some evidence for

379 reproductive senescence (Nussey *et al.* 2013; Zajitschek *et al.* 2019), the drivers of variation

380 in senescence patterns are not fully understood. Here we manipulated both age at mating and

381 nutrition to quantify reproductive senescence in tsetse, a viviparous fly with high maternal

382 allocation and iteroparous reproduction. Both offspring weight and starvation tolerance

383 declined with maternal age, after a peak, yet these patterns of senescence were similar across

384 treatments. Contrary to predictions from life history theory, therefore, neither changes in

385 maternal allocation nor resources affected the timing and rate of reproductive senescence, in

386 terms of offspring quality.

387

388 We did observe a steeper increase in the hazard of mortality with age for nutritionally

389 stressed mothers (S5 File). This suggests that females do not have a survival benefit from

390 reduced reproductive effort, as nutritionally stressed mothers had higher probability of

391 abortion and produced smaller offspring at any age compared with mothers in the control and

392 mating delay groups. This contrasts with findings from studies of other insects where

393 nutritional stress both reduced reproductive output and either maintained or even extended

394 lifespan relative to a control group (De Sousza Santos & Begon 1987; Ernsting & Isaaks

395 1991; Kaitala 1991). Taken together, it may be that factors other than the direct costs of

396 reproduction through physiological damage, or indirectly through resource allocation trade-

397 offs, impose higher mortality rates. Alternatively it may be that, given the extreme maternal

398 allocation in tsetse, even though females produce relatively smaller offspring they still pay a

399 high cost of reproduction in terms of physiological damage; and females on a poor quality

400 diet experience this cost to a greater extent in terms of impact on mortality. This is supported

401   by data from field-caught tsetse, where smaller females invest relatively more of their fat in

402   their offspring, even though their offspring were smaller (Hargrove *et al.* 2018).

403

404   We find that females experiencing nutritional stress have a relatively higher rate of abortion.

405   Hargrove and Muzari (Hargrove & Muzari 2015), using field collected *G. pallidipes*, showed

406   that transfer of the majority of fat to the larva occurs only after c. 80% pregnancy has been

407   completed. Therefore, a female could potentially abort a larva if there are not enough fat

408   reserves for a full-term pregnancy. Evolutionary models tailored to tsetse life-history, with

409   high investment in single offspring across multiple reproductive bouts, could yield insights

410   into whether such spontaneous abortion is an adaptive strategy to retain reserves for future

411   reproduction, or a result of physiological constraints that limit the reserves available

412   (McNamara *et al.* 2009).

413

414   Our study highlights the benefits gained from individual-level data to understand senescence.

415   The bell-shaped relationship of offspring quality with age may have contributed to the

416   relatively small effects of age evident in previous studies where grouped ages and mean

417   values were used, rather than tracking reproductive output from individual females (Langley

418   & Clutton-Brock 1998; McIntyre & Gooding 1998). The bell-shaped pattern observed here is

419   strikingly similar in shape across treatments and reflects the general bell-shaped pattern of

420   reproductive senescence observed across diverse taxonomic groups e.g. (Velando et al. 2006;

421   Sharp & Clutton-Brock 2010). As summarised by (Monaghan *et al.* 2020), a bell-shaped

422   relationship between maternal age and reproductive output, or offspring quality, can arise

423   from population-level effects, due to selective disappearance, but also from individual-level

424   effects. By tracking individual mothers, here we provide evidence that, for tsetse, the bell-

425   shaped relationship between offspring wet weight and maternal age is a consequence of

426   individual effects. The initial increase in offspring wet weight could be a consequence of

427   mothers accruing reserves each time she takes a bloodmeal, so that at each sequential

428   reproductive event she has more energy reserves that can be provided to the offspring. The

429   subsequent decline could then be explained by senescence.

430

431   Tracking individual mothers provided insights into individual variation in maternal

432   allocation. There was marked variation in offspring wet weight among mothers, particularly

433   for the mating delay treatment and variation in senescence patterns for wet weight,

434   particularly for nutritionally stressed mothers. Some females in the mating delay treatment

435   consistently produced smaller than average offspring, across all ages, and these females

436   contributed more to the individual heterogeneity than those producing consistently heavier

437   offspring. For nutritionally stressed mothers, variation between individuals in offspring wet

438   weight increased as mothers aged. These observations suggest that variation in offspring

439   quality is affected not only by mother size but also unmeasured aspects of her condition. The

440   large amount of variation between individuals, in offspring weight and changes in the extent

441   of variation with maternal age in this study was unexpected, suggesting that future studies

442   quantifying the relative roles of mother size and physiological condition on offspring size

443   across different ages would be valuable.

444

445   Offspring from young mothers that were nutritionally stressed had the lowest starvation

446   tolerance. We also found that maternal age affected offspring starvation tolerance

447   independently of age-effects mediated through wet weight, suggesting that there may be other

448   factors associated with maternal age that influence the quality of offspring. More subtle

449   effects of maternal age on the quality of resources transferred to offspring warrant further

450   investigation in tsetse and other species. For tsetse, during late stages of pregnancy females

451   not only transfer fat but also amino acids. It may be that young nutritionally stressed females

452   are limited in these amino acids. (Cmelik *et al.* 1969). The authors reasoned that the tyrosine

453   and phenylalanine obtained from a single bloodmeal is unlikely to be sufficient to meet the

454   amount required by offspring and that a surplus stored from previous bloodmeals may be

455   required. This suggests that size may be limited when nutrients are limited, irrespective of the

456   amount of energy available. It also demonstrates that resource allocation processes are likely

457   more complex, and more nuanced studies on the effects of the quality of resources as well as

458   quantity may be required to understand the ageing process.

459

460   We focused on reproductive senescence in this study. One limitation is that we did not

461   continue the experiment beyond 100 days to quantify more fully mother survival for all three

462   treatments. Our evidence of actuarial senescence in the nutritional stress treatment supports

463   analysis of mark-recapture studies of *G. m. morsitans* in the field (Hargrove *et al.* 2011)

464   which also showed an increase in mortality as a function of age. Considering this, an

465   additional experiment to test whether females mated later experience a delayed onset in

466   actuarial senescence would be informative, given the similar rates of reproductive senescence

467   observed across treatments.

468

469 We also note that our conclusions with respect to reproductive senescence are constrained to

470 this 100-day period. While, in principle, it would be optimal to run the experiment until all

471 females had died, the >1/4 of offspring being aborted and fewer females surviving would

472 result in relatively small sample sizes beyond this time point. However, we are confident that

473 our study timeframe captures an ecologically relevant period to study age-dependent

474 allocation in tsetse, given that field studies show that 90% of females have died before 100

475 days (Hargrove *et al.* 2011). Lastly, we acknowledge that our conclusions may not

476 necessarily extend to wild flies, and further study of tsetse in the field would need to be

477 carried out to confirm this.

478

479 To conclude, our results provide evidence of a bell-shaped relationship between maternal age

480 and offspring quality at the individual-level for the iteroparous tsetse fly. This complements

481 the existing body of work from other species, that has predominantly shown a similar curve

482 for the number of offspring produced as mothers age (Monaghan *et al.* 2020). Contrary to

483 predictions from life history theory, however, our study did not find evidence of a decline in

484 offspring quality as a function of direct costs of reproduction or resource allocation trade-

485 offs.

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604

605   **Figure 1 Overview of experiments.** M – mating. Colour-coded silhouette of pregnant tsetse

606   with larva, timeline shows a silhouette of a pupa and tubes containing blood, the dark red

607   indicates red blood cell and beige indicates serum.

608

609   **Figure 2 Predicted probability of larval abortion as a function of maternal age, by**

610   **treatment.** Predicted probabilities from generalised linear mixed effects model fits to the

611   data and 95% prediction intervals. Plots of raw data are provided in S7 File.

612

613   **Figure 3 Offspring wet weight as a function of maternal age and treatment.** Showing

614   model fits to the data: thick line – population level, thinner lines – individual level. Points –

615   average wet weights for 10-day intervals and 95% confidence intervals. Plots of raw data are

616   provided in S7 File.

617

618   **Figure 4 Effect of sex, wet weight and maternal age on starvation tolerance (the number**

619   **of days a newly emerged fly can survive starvation).** a) Wet weight as a function of

620   offspring sex by treatment; b) Predicted survival time based on linear mixed effects model.

621   Days adults survived starvation is plotted against maternal age. Prediction for each wet

622   weight quartile shown. Plots of raw data are provided in S7 File.

623

624   **Supplementary Files**

625   **S1 File. Pilot data from the tsetse colony at LSTM.**

626   **S2 File. Additional methods.**

627   **S3 File. Nutritional stress pilot.**

628   **S4 Figure. Histogram of offspring survival time by treatment.**

629   **S5 File. Analysis of mother survival.**

630   **S6 File. Statistics.**

631   **S7 File. Raw data plots.**

632   **S8 File. Generalised additive models.**

633   **S9 File. Offspring fat as a function of wet weight.**

634   **S10 File. Statistics excluding mothers in the nutritional stress treatment that died before**

635   **the end of the experiment.**

636