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SPECIAL ISSUE: INSECTS IN PRODUCTION

Effect of temperature on egg production in the common housefly

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Key words: egg-laying rate, oviposition period, temperature-dependent fecundity, rearing condition, *Musca domestica*, Diptera, Muscidae

Abstract

Musca domestica L. (Diptera: Muscidae), better known as the common housefly, is increasingly considered to be a new, alternative protein source for animal nutrition. By transferring low-value organic side streams into high-value protein products, its commercial production contributes to a circular economy. Next to technical innovations for scaling-up the production capacity, efficient egg production has been identified as one of the bottlenecks of housefly production systems. We investigated egg production in two strains, one originally from Spain (SPA) and one from The Netherlands (GK), at 25 and 32 °C. At 25 °C, duration of preoviposition period, laying phase, and adult longevity was longer than at 32 °C. Lifetime egg production was lower at 32 °C, but the number of clutches laid per female was unaffected by temperature. Daily egg production at 32 °C was higher during the first 7 days, revealing a trade-off between higher early-in-life reproductive effort and adult longevity. The combination of shorter sexual maturation period and higher daily egg-laying rate resulted in reaching 50% of total egg production only 6 days after emergence at 32 °C for both strains, compared to 13 and 14 days at 25 °C for SPA and GK, respectively. We conclude that, in the absence of a need for high adult survival rates, houseflies have favourable production performances at higher temperature, and that efficacy and yield of the production process could be maximized by increasing the rearing temperature to 32 °C.

Introduction

Insects are considered to be a new, alternative protein source for animal nutrition (Sánchez-Muros et al., 2014) while contributing to a circular economy by transferring low-value organic side streams into high-value protein products. The emerging insect industry could be one of the ways to enhance food and feed security (Al-Qazzaz et al., 2016) and to reduce negative ecological effects of greenhouse gasses and ammonia emissions of traditional livestock (Oonincx et al., 2010). *Musca domestica* L. (Diptera: Muscidae), better known as the common housefly, is increasingly being considered as a protein source for livestock feed, and its commercial production may aid in waste management. The high content of proteins and fat,

low level of fibres, and the amino acid composition matching the ideal feed composition for poultry make housefly-based feed comparable to the most common protein sources in poultry nutrition such as soybean meal, fish, and meat-bone meal (Gadzama & Ndudim, 2019). Easy rearing requirements and the ability of housefly larvae to grow on many different substrates make them useful to turn waste (e.g., manure) into a valuable biomass (Pastor et al., 2011; Čičková et al., 2012; Zhang et al., 2012).

Although it is a highly proliferous insect species and can be cultured at large-scale throughout the year, industrialization of housefly rearing faces numerous challenges. Next to technical innovations for scaling-up production capacity (Čičková et al., 2015), biological parameters may be optimized. Because rearing at high density under mass-rearing conditions could have a detrimental effect on reproduction and fecundity of houseflies, efficient egg production has been identified as one of bottlenecks of production systems (Pastor et al., 2011, 2015). Additional factors, including environmental temperature (Fletcher

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et al., 1990), oviposition substrate (e.g., Ahmed et al., 2013), quality and quantity of diet (Shipp & Osborn, 1967), and genetic properties of the strain (Zvereva & Zhemchuzhina, 1988) have been shown to affect housefly oviposition and lifetime egg production. Moreover, trade-offs may exist between egg production and other life-history traits. For example, although a considerable amount of literature has been published on egg-laying behaviour and egg production of houseflies, this knowledge has scarcely been applied to mass-rearing conditions. Commercial production requires new approaches to enhance early-in-life oviposition, to continually produce large numbers of eggs, and to identify trade-offs that are specific for mass-rearing conditions.

Temperature influences ectotherm biology at multiple levels, from physiology and stress tolerance to life-history traits and fitness. In a considerable number of insect species, including *M. domestica*, higher than usual (optimal) temperatures increase metabolic functions, influence developmental rate, longevity, body size, locomotor and mating activity, and reproductive output (e.g., Fletcher et al., 1990; Berger et al., 2008). For houseflies, the amplitude of daily thermal fluctuations might vary by season and habitat, with peak temperatures exceeding 30 °C (Koné et al., 2017). Its broad geographical distribution, wide ecological amplitude, and large population sizes indicate that houseflies cope with elevated temperatures successfully (Lysyk & Axtell, 1987). Like other insects, they may also display acclimation responses to increased temperature, such as shorter sexual maturation period (Michelsen, 1960), shorter preoviposition period (Larsen & Thomsen, 1940), shorter interval between successive egg batches (Buéi, 1959), and higher daily reproductive output at temperatures over 30 °C (Dunn, 1922). In addition, their plasticity in behavioural responses and resistance to temperature stress (Kjærsgaard et al., 2015) indicate that culturing houseflies at increased temperatures should be given more attention.

Despite its relevance, the role of *M. domestica* thermal response (range) to suboptimal temperatures has not been regularly included in experimental life-history studies. For example, most of the fecundity tests under laboratory conditions were performed at room temperature (around 20 °C) or/and at 25 °C (Greenberg, 1955; Khan et al., 2012; Ahmed et al., 2013), which is considered to be an optimal temperature for housefly culturing. Moreover, published protocols on large-scale housefly production also included rearing conditions at 25 °C only (Pastor et al., 2011; Čičková et al., 2012; Wang et al., 2013). Next to fecundity data, limited information is also available on temperature-driven effects on the full range of life-history attributes, fitness trade-offs, and their practical application

for housefly mass rearing. As industrialization requires maximal values of beneficial production traits, trade-offs relevant under natural conditions may become irrelevant in the selective environment that flies experience in mass-rearing facilities.

This paper aims to contribute to improvement of the conditions (i.e., temperature) under which housefly adults are maintained in order to increase the effectiveness of the rearing process. Specifically, by testing life-history and egg-production traits of two strains, one originally from a Mediterranean climate (Spain) and one from a more temperate climate (The Netherlands) at two temperatures (25 and 32 °C), we aimed to gain more insight into optimization of housefly production systems.

Materials and methods

Houseflies were collected in 2015 in Spain from the regions of Girona (three locations: Calonge, St. Jordi Desvalls, and Riudellots de la Selva) and Barcelona (two locations: Cerdanyola del Vallès and Sant Cugat del Vallès). More detailed information on sampling points are summarized in Table S1. Specimens were identified based on characteristics defined by d'Assis-Fonseca (1968) and they had been in separate culture for about 4 years before initiating this study. The flies were kept in a climate room at a constant 25 °C, 50 ± 10% r.h., and L10:D14 photoperiod. In 2018, 500 flies – 50 females and 50 males of each strain – were pooled to generate an outbred experimental strain from Spain (SPA). Six strains of houseflies originating from wild-caught individuals collected in June 2018 from Gerkesklooster, The Netherlands, had been bred separately in the laboratory for eight generations at 25 °C, before approximately 1 000 adult flies (about 80 females and 80 males flies of each strain) were pooled to establish a genetically variable Dutch experimental strain (GK). After their initiation, both the GK and SPA strains were cultured in high numbers (>5 000 individuals) for three generations to allow for genetic mixing and recombination, and thereafter experiments were started at 25 °C. Subsequently, 4 000 adult flies from each of the GK and SPA strains were transferred from 25 °C to an incubator at a constant 32 °C, 50 ± 10% r.h., and L10:D14 photoperiod, and maintained at 32 °C for five generations until used for experiments. Adult flies were fed water, 20% sucrose solution, and milk-powder, whereas larvae were reared on a diet according to Hilfiker-Kleiner et al. (1994) with slight modifications: 1 250 ml of water, 20 ml ethanol containing 0.67 g Nipagin, 1 000 g wheat bran, 150 g wheat flour, 120 g milk powder, and 50 g brewer's yeast. Potential differences in microbial communities and nutritional properties of the feed between the two rearing

conditions were not evaluated. Larval densities were not controlled in detail but remained approximately constant (four eggs g^{-1} of feed) for the duration of the experiment.

After emergence from the pupae, individual males and females were paired, and transferred to plastic 180-ml oviposition containers. In total, 226 pairs were included in the experiment – 53 pairs each of the GK and SPA strains cultured at 25 °C, and 50 and 70 pairs reared at 32 °C, respectively. Each of the oviposition chambers contained a small cotton pad soaked with milk in a disposable Petri dish to allow for oviposition, along with milk powder and 20% sucrose solution for adult nutrition. The cotton pads were replaced daily and the number of eggs produced during that 24-h period was recorded. Fecundity data were collected by removing the eggs from the cotton pad with a soft brush, examining the pad to assure that all eggs were taken, and counting the eggs under a stereomicroscope. Hatched eggs were also counted. Daily egg production records were kept for each pair, and the experiment was continued until death of all flies. Oviposition lasted until the end of housefly life and no post-oviposition period was observed. Females continued to be monitored for reproduction and mortality after death of the male, and males were not replaced. Mild CO₂ anaesthesia was used during the experiment to change cotton pads.

The following life-history parameters were determined for all individuals: (1) preoviposition period (time between emergence of an adult female and the onset of her first oviposition), (2) oviposition period (time from first egg batch until death of an individual), (3) lifetime egg production (total number of eggs produced during an individual's life), (4) number of clutches, and (5) average daily egg production (lifetime egg production divided by number of days of the oviposition period).

Statistical analysis

Descriptive statistics including mean, standard deviation, and maximal values were calculated for the size of the first clutch, total number of clutches, daily and cumulative egg production, duration of preoviposition period, laying phase, and overall longevity. Normality of the data was assessed with the Shapiro-Wilks test. Daily egg production data were log-transformed to meet the assumptions of normality. Continuous variables (lifetime egg production, egg production per day, and longevity of adult flies) were analysed by general linear model (GLM) with 'temperature' (25 and 32 °C), 'strain' (GK and SPA), and 'sex' (included only in longevity test) as predictor variables. The possible interactions between the variables were considered in GLM models, and followed by pairwise Welch's

t-tests. We applied GLM procedure to run regressions and explore the relation between female total lifetime egg production and size of the first clutch for each strain and each temperature treatment independently. Count data (number of clutches per female) were analysed with generalized linear model with Poisson distribution, log link, and 'temperature' and 'strain' as factors. Statistical analyses were performed in STATISTICA v.13.3 (TIBCO Software, Palo Alto, CA, USA).

Results

First oviposition and frequency of oviposition

In total, the 226 pairs produced 88 845 eggs. At 25 °C, the preoviposition period lasted on average 6 days, when first egg clutches were observed for 23% GK and 34% SPA females (Table 1). Most females (51% GK, 60% SPA) laid their first eggs on day 7, and only a small proportion of females started their egg production on days 8–12 (Figure 1). In contrast, only 2 days were needed for sexual maturation of houseflies cultured at 32 °C (Table 2), when 68% of GK females and 49% of SPA females deposited their first egg batches. Most other females started laying eggs on day 3 (22% GK, 33% SPA), and by day 5, all GK and SPA females had deposited their first clutches at 32 °C (Figure 1).

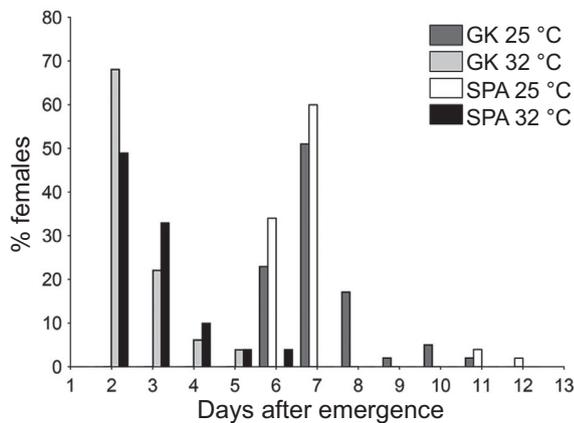
Houseflies at the lower rearing temperature had a lower egg-laying rate. Females of both strains oviposited on average every 3–5 days, and the mean total duration of the laying phase was 16.4 and 17.5 days for SPA and GK, respectively (Table 1, Figure 2). At 32 °C, the period between two oviposition bouts was shorter. Females of both GK and SPA deposited eggs daily or every other day in the 1st week, and thereafter the frequency of oviposition was every 2–3 days. The length of the oviposition period also decreased at the higher rearing temperature to only 10.53 and 11.34 days for SPA and GK, respectively. Still, although the interval between successive egg batches was shorter at 32 °C, the total number of egg clutches laid per female was unaffected by temperature ($\chi^2 = 0.472$, d.f. = 1, $P = 0.49$) and strain ($\chi^2 = 4.758$, d.f. = 1, $P = 0.057$).

Lifetime and daily egg production

At 25 °C, high numbers of eggs were collected for both strains from the end of the 1st week (days 6–7), and this number remained rather stable during the following 2 weeks (days 8–21). A decrease in egg production was observed during the 4th and 5th weeks for both strains, whereas GK retained a low level of oviposition in the 6th week (days 36–39). The maximum number of eggs was produced on day 7 for SPA and on day 11 for GK, and

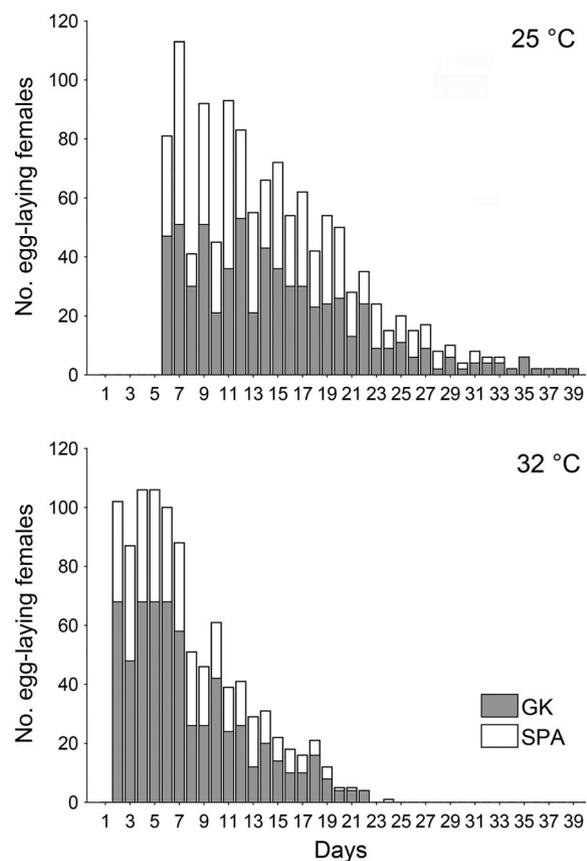
Table 1 Mean (\pm SE) and maximum (in parentheses) of adult longevity (days) and egg production parameters of two *Musca domestica* strains (GK and SPA) at 25 and 32 °C

Parameter	GK		SPA	
	25 °C	32 °C	25 °C	32 °C
Sample size (pairs)	53	50	53	70
Preoviposition period (days)	6.26 \pm 0.15 (10)	1.46 \pm 0.11 (4)	5.94 \pm 0.17 (11)	1.83 \pm 0.13 (5)
Oviposition period (days)	17.53 \pm 1.11 (35)	11.34 \pm 0.81 (23)	16.42 \pm 1.10 (29)	10.53 \pm 0.64 (22)
Total no. eggs per female	491 \pm 35.41 (1175)	398 \pm 26.24 (735)	405 \pm 32.02 (1009)	306 \pm 16.43 (571)
Daily egg production per female (no. eggs)	31.01 \pm 1.36 (51.5)	43.81 \pm 2.29 (119)	28.97 \pm 1.86 (90)	37.35 \pm 1.69 (88)
No. clutches per female	6.17 \pm 0.36 (11)	6.28 \pm 0.47 (13)	5.77 \pm 0.39 (11)	5.25 \pm 0.29 (9)
Size of the first clutch (no. eggs)	77.23 \pm 3.10 (129)	70.98 \pm 2.58 (119)	72.83 \pm 2.38 (117)	74.46 \pm 1.43 (98)
Longevity of females (days)	23.87 \pm 1.08 (41)	12.80 \pm 0.79 (24)	22.36 \pm 1.07 (35)	12.34 \pm 0.64 (25)
Longevity of males (days)	22.98 \pm 1.04 (39)	11.22 \pm 0.79 (24)	21.66 \pm 1.02 (34)	11.27 \pm 0.71 (24)

**Figure 1** Temporal distribution (%) of egg-laying females in two *Musca domestica* strains (GK and SPA) depicting day of first oviposition at 25 and 32 °C.

50% of total egg production at 25 °C was reached 13 and 14 days after emergence for SPA and GK, respectively. In contrast, the 1st week was the most productive week at 32 °C, when high numbers of eggs were laid on days 2–7 for both strains. The peak of oviposition occurred on day 2 for SPA and on day 6 for GK, and 50% of total egg production at 32 °C was already reached 6 days after emergence for both strains (Figure S1).

Temperature ($F_{1,222} = 12.345$) and strain ($F_{1,222} = 10.418$, both $P < 0.001$), but not their interaction ($F_{1,222} = 0.009$, $P = 0.92$), significantly influenced lifetime egg production. Higher temperature resulted in decreased egg production in the GK strain ($t = 2.127$, d.f. = 94.54, $P = 0.036$) with an average reduction of 103 eggs per female. Similarly, overall fecundity for the SPA strain was reduced at higher temperature by 99 eggs ($t = 2.749$, d.f. = 78.873, $P = 0.007$). SPA had lower total egg production than GK at elevated temperature ($t = 2.943$,

**Figure 2** Daily variation in egg laying of two *Musca domestica* strains (GK and SPA) at 25 and 32 °C.

d.f. = 85.616, $P = 0.004$), but these differences were not significant at 25 °C ($t = 1.799$, d.f. = 102.965, $P = 0.075$) (Figure 3).

The size of the first clutch was a good predictor of the total number of eggs produced by a female during her life

Table 2 Overview of literature data on lifetime egg production (mean + range in parentheses) of *Musca domestica* as function of temperature

Temperature (°C)	Experimental details					Adult feed	Larval feed	Oviposition substrate	Reference
	20	25	26	27	30				
-	-	405 (71–1009)	-	-	-	20% sucrose solution, milk-powder, and water	Wheat bran, wheat flour, milk powder, brewer's yeast, Nipagin, and water	Cotton pad soaked with milk	This study
-	-	491 (32–1175)	-	-	-	20% sucrose solution, milk-powder, and water	Wheat bran, wheat flour, milk powder, brewer's yeast, Nipagin, and water	Cotton pad soaked with milk	This study
117.8	433.1	-	-	494.9	-	Diluted evaporated milk and granulated sucrose	Mixture of CSMA medium ¹ , yeast, and water	Cotton pad soaked with milk	Fletcher et al. (1990)
-	(39–2387)	-	-	-	-	Milk	Horse manure	Horse manure	Dunn (1922)
-	826	-	-	-	-	Powdered milk and honey	No information	Cellucotton moistened with a mixture of powder milk and honey	Greenberg (1955)
-	(290–447)	-	-	-	-	Sugar, milk powder, and water	Pig manure	Pig manure	Pastor et al. (2011)
-	(324.7–381.4)	-	-	-	-	Sugar, milk powder, and water	Pig manure	Pig manure	Pastor et al. (2011)

SPA strain: based on individual pairs kept at constant temperature of 25 and 32 °C

GK strain: based on individual pairs kept at constant temperature of 25 and 32 °C

Based on cage estimates (at each temperature the total no. eggs laid divided by the initial no. females)

Outdoor conditions of January–March in Panama (average mean, min, and max daily temperature: 25, 21, and 31 °C)

Based on individual pairs under laboratory conditions

Egg production at different adult densities (14.2 and 56.8 cm³ space available per fly at min and max egg production, respectively)

Evaluation of adult size (correlated with pupal weight) on mean egg production per female (mean weight of pupae 10.523 and 20.840 mg for min and max egg production, respectively)

Table 2 Continued

Temperature (°C)							Experimental details	Adult feed	Larval feed	Oviposition substrate	Reference
20	25	26	27	30	32	35					
-	(307-443)	-	-	-	-	-	Tested on different livestock manures	Sugar, powdered milk, and water	Livestock manure	Mixture of bran, grass meal, yeast, sugar, powdered milk	Khan et al. (2012)
-	(242.4-259)	-	-	-	-	-	Room temperature; addition of <i>Dodonia viscosa</i> (L.) Jacq. extract (oviposition attractant)	Granulated sugar and water	Brewer's yeast, milk powder, wheat bran, and water	Larval medium with plant extract	Ahmed et al. (2013)
-	-	(90-288)	-	-	-	-	Tested on diets with different protein sources	Granulated sucrose and protein ingredients including skim milk, cream milk, powdered eggs, and yeast	Bran, full-cream milk powder, Nipagin, and water	Cotton wool saturated with 5% solution of full-cream powdered milk	Shipp & Osborn (1967)
-	-	-	390	-	-	-	Test of weightlessness on egg production (performed after space flight)	Evaporated milk and solution consisting of 12.1% sucrose, 8% glucose, and 0.9% fructose	CSMA medium ¹	CSMA medium ¹	Lee et al. (1985)
-	-	-	365.2	-	-	-	Based on individual pairs	Diluted evaporated milk	CSMA medium ¹	CSMA medium ¹	Reed & Bryant (2004)
-	-	-	(267-700)	-	-	-	Based on individual pairs	Diluted milk powder	No information	No information	Feldman-Muhsam (1944)

¹CSMA medium: alfalfa meal 27%, wheat bran 33%, brewers' dried grains 40% (Ralston-Purina, St. Louis, MO, USA).

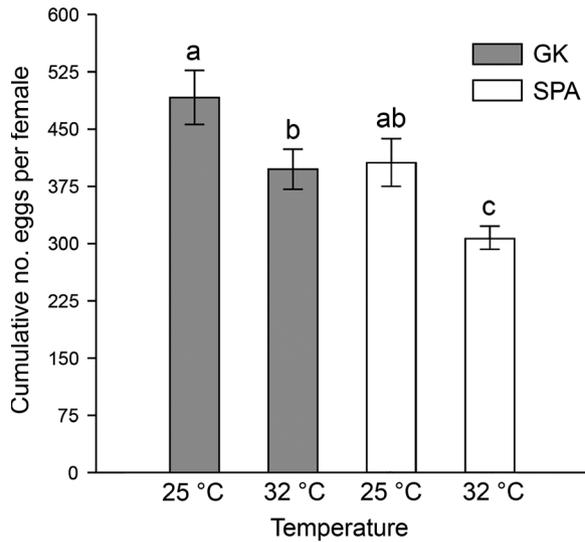


Figure 3 Mean (\pm SE) lifetime egg production of two *Musca domestica* strains (GK and SPA) at 25 and 32 °C. Means capped with different letters are significantly different (pairwise Welch's t-tests: $P < 0.05$).

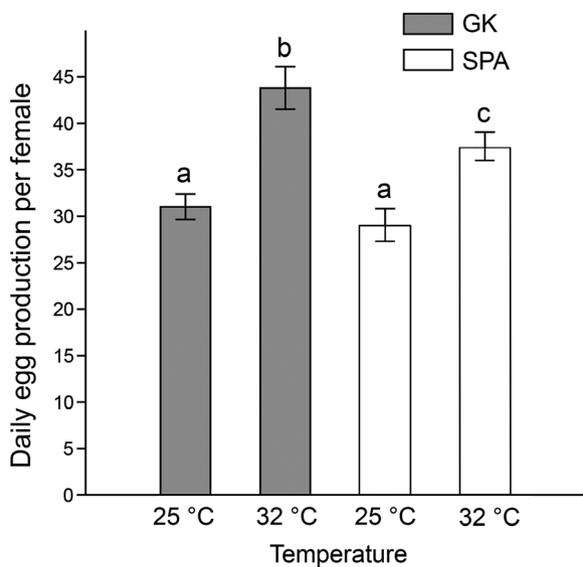


Figure 4 Mean (\pm SE) daily egg production of two *Musca domestica* strains (GK and SPA) at 25 and 32 °C. Means capped with different letters are significantly different (pairwise Welch's t-tests: $P < 0.05$).

for the GK strain reared at 25 °C ($F_{1,51} = 21.316$, $P < 0.01$; $r^2 = 0.543$) and for the SPA strain at 32 °C ($F_{1,68} = 14.188$, $P < 0.01$; $r^2 = 0.415$). However, the positive correlation between the size of the first clutch and overall egg production was not significant for GK at higher ($F_{1,48} = 0.629$, $P = 0.43$; $r^2 = 0.114$) and SPA at lower ($F_{1,51} = 2.904$, $P = 0.094$; $r^2 = 0.232$) rearing temperatures.

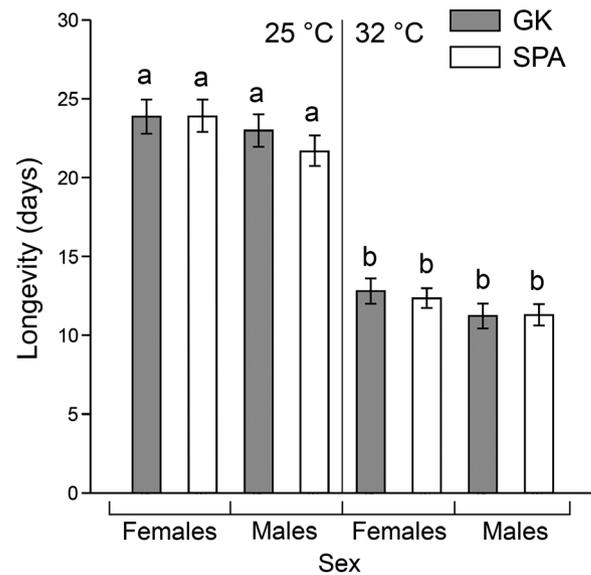


Figure 5 Mean (\pm SE) longevity (no. days) of two *Musca domestica* strains (GK and SPA) at 25 and 32 °C. Means capped with different letters are significantly different (pairwise Welch's t-tests: $P < 0.05$).

Daily egg production was affected by temperature ($F_{1,222} = 33.363$, $P < 0.001$) and strain ($F_{1,222} = 5.374$, $P < 0.05$), but not by their interaction ($F_{1,222} = 1.444$, $P = 0.23$). Higher temperature resulted in increased daily egg production for the SPA strain. SPA females laid on average 28.97 eggs per day of adult life at 25 °C, whereas mean daily egg production at 32 °C (37.35 eggs per day) was higher ($t = 4.139$, d.f. = 108.859, $P < 0.001$). Similarly, GK flies cultured at 32 °C laid about 12 more eggs per day than flies bred at 25 °C ($t = 5.262$, d.f. = 100.936, $P < 0.001$). Daily egg production at 25 °C was comparable for GK and SPA ($t = 1.253$, d.f. = 103.767, $P = 0.21$), but at 32 °C SPA females produced fewer eggs per day ($t = 2.272$, d.f. = 110.353, $P = 0.007$) (Figure 4).

Longevity

No sex differences in longevity were apparent in the GK and SPA strains (Table S2), and lifespan of both sexes of both strains decreased with increasing temperature (Figure 5). In addition, there was no interaction among these three factors (Table S3). Thus, a trade-off between adult longevity and early-in-life reproductive effort was revealed.

Discussion

The current study investigated the impact of temperature (25 and 32 °C) on egg production of the housefly in the laboratory. Both analysed strains responded to different

rearing conditions with altered duration of preoviposition period, laying phase, and overall longevity. Moreover, higher daily egg production at 32 °C was observed, but this resulted in lower survival. Hence, a trade-off between adult longevity and early-in-life reproductive effort was evident. Such life-history trade-offs are known from many organisms (Bell, 1980; Roff, 1992). Flies cultured at 32 °C began laying eggs about 4 days earlier and had significantly higher daily fecundities than those laying at 25 °C. The SPA flies that laid at 32 °C averaged about eight more eggs per day than flies that developed at 25 °C, whereas daily egg production for GK was on average 12 eggs higher at higher rearing temperature. Temperature also had a significant effect on overall fecundity of both housefly strains, both SPA and GK females laid on average 100 eggs fewer at 32 °C (19 and 25% for GK and SPA, respectively). Although elevated temperature negatively affected the fecundity of individual flies, the overall efficiency of the rearing process was increased. Egg production at 32 °C started 4 days earlier and laying phase was on average 6 days shorter, meaning a sizeable reduction in duration of the egg collecting process compared to that at 25 °C. A shorter sexual maturation period together with higher daily egg-laying rate resulted in reaching 50% of total egg production only 6 days after emergence (in comparison to 13 and 14 days at 25 °C for SPA and GK, respectively), which is the major advantage of housefly culturing at 32 °C. As the rate at which eggs mature within the ovaries is largely dependent on temperature (Elvin & Krafur, 1984), our results verify the favourable reproductive output and beneficial effects on production performances of *M. domestica* at a temperature higher than 25 °C, which is often considered as the optimal temperature for development and oviposition (Pastor et al., 2011, 2015).

Our interpretation of the above patterns may have been confounded by several issues. Firstly, because we did not determine the proportion of eggs laid that produced adult offspring, our cumulative egg counts could possibly overestimate the actual number of offspring per female, even ignoring normal mortality between egg and adult. Still, in a pilot experiment, the hatchability, percentage of pupation, and adult emergence rate were comparable at both temperature treatments and for both strains, therefore they were independent of developmental temperature (L. Francuski, unpubl.). Secondly, paternal effects on egg production should not be ignored. Michelsen (1960) suggested that temperature of 34 °C might cause a decreasing fertility and (partial) sterilization of males, although available data at 35–37 °C demonstrated no sterilizing effect (Saccá & Benetti, 1958; Buéi, 1959; Fletcher et al., 1990), and from our data it does not seem to be the case at 32 °C either. It is unclear whether female and male *M. domestica*

have different thresholds or responses to elevated temperature, and this requires further investigation.

Although temperature and humidity have long been considered as relevant factors, most insect rearing has been done at a single, constant temperature (Cohen, 2018). To our knowledge, housefly lifetime egg production has never been considered at a laboratory condition of 32 °C (see literature overview in Table 2). The only comparable study of Fletcher et al. (1990) reported the mean number of eggs laid per female at 30 (494.9 eggs) and 35 °C (365.5 eggs), and suggested that houseflies can reproduce over a temperature range of 20–35 °C. Their reported fecundities correspond well with our study (405 and 491 eggs at 25 °C and 306 and 398 eggs at 32 °C for SPA and GK, respectively). High densities in nature, wide ecological amplitude, and continuing presence of active populations during the warm months of the year indicates that not only houseflies may have substantial tolerance to suboptimal temperatures, but also that the threshold temperature is likely to be above 32 °C outdoors. Nevertheless, increase in temperature to 35 °C resulted in noticeable reduction in female productivity under laboratory conditions (Fletcher et al., 1990). Moreover, in the tropics when the average maximum temperatures reach 38–40 °C and relative humidity decreases in hot seasons, adult flies are less numerous and less active in the natural environment (Keiding, 1986; Koné et al., 2017). As fecundity is a major determinant of female fitness (e.g., Roff, 1992), knowing the upper thermal limit of egg laying is important for improving the efficiency of the housefly rearing process. Based on published results (Fletcher et al., 1990) and our data, culturing conditions at temperature range 30–32 °C seem well suited for efficient housefly rearing. As higher rearing conditions resulted in lower survival, a favourable output in mass rearing facilities may be reached at 32 °C, when flies are replaced at a regular high frequency. Evaluation of the effects of elevated temperature on housefly rearing also requires knowing the flexibility and costs of the acclimation responses. In addition, thermal responses should be measured at all (sensitive) life stages, including eggs, larvae, and pupae, across multiple fitness traits and generations (Hoffmann et al., 2013) and across different side streams. Next to temperature, fecundity is affected by many other abiotic factors (e.g., humidity, nutrition of immature stages and adults) and biotic factors (e.g., larval and adult density, adult size), which need to be studied further to improve housefly mass production. Our future research will focus on all fitness components relevant for mass production, including growth, survival, mating, fertility, and other traits.

Although not all strain differences were statistically significant, SPA had consistently lower values of egg production and shorter life span than GK at both temperatures.

Temperature-dependent differences in individual responses are frequently explained by the thermal adaptation hypothesis which states that, for wide-ranging species that experience different thermal gradients in different environments, populations may exhibit variation in thermal adaptations and responses to suboptimal temperatures (Sunday et al., 2012). This may lead to local adaptation meaning that populations inhabiting warmer regions will function better at higher temperatures relative to populations from cooler locations and vice versa (Angilletta et al., 2003). Although it seems that life-history traits and fitness of the northern (Dutch) strain were less affected by elevated rearing temperature than the southern (Spanish) strain, it must be noted that Spanish flies had been cultured for 3 years (about 75 generations) longer in the laboratory at 25 °C than the GK strain (6 months after collection, 11 generations of laboratory culturing). We pooled five Spanish strains to generate an outbred experimental strain at the start of the experiment, but this may not have prevented effects of genetic drift, inbreeding, and selection after initial colony establishment. Still, our results point towards variation between strains in their adaptation to rearing conditions which motivates testing more populations of different geographical origin. In addition, as genetic variation for temperature-dependent fecundity in populations is likely (Prasad et al., 2010), artificial selection for increased adaptation to high production conditions may be an important step for future commercialization. Regardless of the rearing temperature, mass culturing of the common housefly still involves many areas which have not been sufficiently surveyed (Čičková et al., 2012) and establishment of protocols that can prevent beneficial trait decline is highly needed. Our experiment indicated that it is indeed feasible to maximize efficiency and yield by increasing the rearing temperature, and our future research will focus on verifying these results in medium- and large-scale housefly production systems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Cumulative number of eggs depicting variation in lifetime egg production between two *Musca domestica* strains (SPA and GK) at 25 and 32 °C. Arrows indicate the days when 50% of total egg production was reached – 6 days after emergence for SPA and GK at 32 °C, 13 and 14 days at 25 °C for SPA and GK, respectively.

Table S1. Collection sites of housefly strains.

Table S2. Pairwise differences in longevity between the strains, temperature treatments, and sexes. Welch's t-values are given below the diagonal and P-values above the diagonal.