# Thermal phenotypic plasticity of pre- and post-copulatory male harm buffers sexual conflict in wild *Drosophila melanogaster*

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**ABSTRACT**

Strong sexual selection frequently leads to sexual conflict and ensuing male harm, whereby males increase their reproductive success at the expense of harming females. Male harm is a widespread evolutionary phenomenon with a strong bearing on population viability. Thus, understanding how it unfolds in the wild is a current priority. Here, we sampled a wild *Drosophila melanogaster* population and studied male harm across the normal range of temperatures under which it reproduces optimally in nature by comparing female lifetime reproductive success and underlying male harm mechanisms under monogamy (i.e., low male competition/harm) vs. polyandry (i.e., high male competition/harm). While females had equal lifetime reproductive success across temperatures under monogamy, polyandry resulted in a maximum decrease of female fitness at 24°C (35%), reducing its impact at both 20°C (22%), and 28°C (10%). Furthermore, female fitness components and pre- (i.e., harassment) and post-copulatory (i.e., ejaculate toxicity) mechanisms of male harm were asymmetrically affected by temperature. At 20ºC, male harassment of females was reduced, and polyandry accelerated female actuarial ageing. In contrast, the effect of mating on female receptivity (a component of ejaculate toxicity) was affected at 28ºC, where the mating costs for females decreased and polyandry mostly resulted in accelerated reproductive ageing. We thus show that, across a natural thermal range, sexual conflict processes and their effects on female fitness components are plastic and complex. As a result, the net effect of male harm on overall population viability is likely to be lower than previously surmised. We discuss how such plasticity may affect selection, adaptation and, ultimately, evolutionary rescue under a warming climate.

## INTRODUCTION

Females and males share the common goal of siring offspring. This central tenet of sexual reproduction enforces a certain degree of cooperation between the sexes. However, anisogamy frequently leads to distinct sex roles and thus general asymmetries in the reproductive evolutionary interests of females and males, which can in turn result in diverging intensity and form of sexual selection across the sexes (Arnqvist & Rowe, 2005; Chapman, Arnqvist, Bangham, & Rowe, 2003; Janicke, Häderer, Lajeunesse, & Anthes, 2016; Winkler, Moiron, Morrow, & Janicke, 2021). This phenomenon, termed sexual conflict, favours traits in one sex that might be costly for the other (Parker, 1979), and can thus lead to antagonistic female-male coevolution (Arnqvist & Rowe, 2005). Sexually antagonistic co-evolution has received much attention, and is recognized as a fundamental process in evolution due to its role in shaping male and female adaptations (Bonduriansky, Maklakov, Zajitschek, & Brooks, 2008), in contributing to drive reproductive isolation and speciation (Arnqvist, Edvardsson, Friberg, & Nilsson, 2000; Bonduriansky, 2011; Bonduriansky & Chenoweth, 2009; Gavrilets, 2014), and as a major determinant of population demography (Kokko & Brooks, 2003; Bonduriansky & Chenoweth, 2009; Berger et al., 2016). Specifically, sexual conflict has been shown to have profound consequences for female fitness and population growth when it favours male reproductive traits that increase male intra-sexual competitive ability at the expense of harming females (i.e., male harm, Crudgington & Siva-Jothy, 2000; Gómez-Llano, García-Roa, Noble, Faria, & Carazo, 2023; Wigby & Chapman, 2004).

Harmful male adaptations are widespread and incredibly diverse and sophisticated across the tree of life (Arnqvist & Rowe, 2005). For example, male harassment of females during pre-copulatory competition for mating has been documented in myriad vertebrate and invertebrate species (Gómez-Llano et al., 2023), driving antagonistic female-male co-evolution in a host of behavioural and morphological traits (Arnqvist & Rowe, 2005). Male harm adaptations in the context of post-copulatory competition are similarly widespread in invertebrates, featuring (amongst others) toxic ejaculates (Wigby & Chapman, 2005), love darts (Koene & Schulenburg, 2005), and a range of male adaptations for traumatic insemination that range from genital ablation to spiny penises (Crudgington & Siva-Jothy, 2000; Lange, Reinhardt, Michiels, & Anthes, 2013). Importantly, beyond driving female and male phenotypes and associated diversification processes, male harm generally leads to a "reproductive tragedy of the commons" that can substantially impact population demography

by depressing net female productivity (Arnqvist & Tuda, 2010; Berger et al., 2016; Holland & Rice, 1999; Rankin et al., 2011), and even facilitate population extinction (Le Galliard, Fitze, Ferriere, & Clobert, 2005). Understanding what factors underlie male harm evolution, its diversity in form, strength, and outcomes, is thus a main concern in evolutionary biology.

Despite a growing number of studies in the field of sexual conflict, most have been conducted under uniform laboratory conditions, frequently in populations adapted to stable environments for many generations (Chapman et al., 2003b; Hopkins et al., 2020; Wigby & Chapman, 2004, 2005). In contrast, recent research has highlighted the role of ecology in shaping the evolution of traits under sexual conflict (Arbuthnott et al., 2014; García-Roa et al., 2019; MacPherson et al., 2018; Perry & Rowe, 2018; Yun et al., 2017), including habitat complexity (Malek & Long, 2019; Miller & Svensson, 2014; Myhre, Forsgren, & Amundsen, 2013), nutritional status (Fricke, Bretman, & Chapman, 2010), or sex ratio and population density (Chapman et al., 2003a). For example, Gomez-Llano and colleagues (2018) recently showed that conspecific densities and the presence of heterospecifics modify the intensity and outcome of sexual conflict in the banded demoiselle (*Calopteryx splendens*), and the spatial complexity of the environment in which mate competition occurs mediates how sexual conflict operates in fruit flies (Yun et al., 2018). The incorporation of more realistic ecological scenarios in the study of sexual conflict is thus a key avenue to disentangle the evolution of male harm, and its consequences for population viability (Cornwallis & Uller, 2010; Fricke, Perry, Chapman, & Rowe, 2009; Plesnar-Bielak & Łukasiewicz, 2021).

Temperature is recognized as a crucial abiotic ecological factor due to its impact on life history traits and physiological and behavioural responses (De Lisle, Goedert, Reedy, & Svensson, 2018; Kim, Jang, & Lee, 2020; Miler, Stec, & Czarnoleski, 2020; Monteiro et al., 2017). Furthermore, temperature varies in nature widely within and across spatio-temporal scales (e.g., daily, inter-seasonal and intra-seasonal variation). Consequently, it may have short, medium, and long-term effects on organism phenotypes that can impact many different aspects of its reproductive behaviour (e.g., sex-specific potential reproductive rates, operational sex ratios, density, etc.; García-Roa et al., 2020). In fact, a recent meta-analysis suggests that temperature may have a sizeable effect on sexual selection processes even when fluctuations occur well within the normal range of temperature variation for the studied species (García-Roa et al., 2020). This latter finding is particularly relevant given that we know almost nothing about how average temperature fluctuations, such as those

experienced by wild populations during their reproductive season, affect male harm and sexual selection at large.

Our aim was to contribute to fill this gap in knowledge by studying how male harm responds to temperature variation that mimics average fluctuations that are normal during the reproductive season, using *Drosophila melanogaster* flies sampled from a wild population. *D. melanogaster* is an ideal subject for this study because it exhibits high levels of male-male competition, has well characterized pre- and post-copulatory male harm mechanisms, and is perhaps the main model species in the study of sexual conflict (Arbuthnott et al., 2014; Chapman et al., 2003a; MacPherson et al., 2018; Malek & Long, 2019; Wigby & Chapman, 2005). During male-male pre-copulatory competition over access to mating, males harm females via intense harassment that causes physical injuries, interferes with female behaviours such as egg-laying or feeding, and results in energetically costly resistance to males (Bretman & Fricke, 2019; Partridge & Fowler, 1990; Teseo, Veerus, Moreno, & Mery, 2016). In the context of sperm competition over fertilizations, males can also harm females via toxic ejaculates, whereby certain male seminal fluid proteins manipulate female re-mating and egg-laying rates to the male's advantage, but at a costs to females in terms of lifespan and lifetime reproductive success (Chapman et al., 2003b; Sirot et al., 2009; Wigby & Chapman, 2005). These proteins are secreted by male accessory glands, have been well characterised, and are strategically allocated by males in response to variation in the socio-sexual context (Hopkins et al., 2019; Sirot, Wolfner, & Wigby, 2011). Additionally, despite the fact that almost all work on male harm has, to date, been conducted in laboratory strains adapted to stable temperature conditions, *D. melanogaster* reproduces in the wild under a wide range of temperatures (Dukas, 2020; Kapun et al., 2018).

Briefly, we collected flies from a continental wild population in Requena (Spain) that experiences significant fluctuations in temperature even during the mildest months when it is reproductively active (e.g., July: average: 24.9°C, average min: 19.8°C, average max: 30.1°C, Fick & Hijmans, 2017). After acclimation of the resulting population to laboratory conditions under a fluctuating temperature regime mimicking natural conditions, we conducted 5 different experiments to gauge how temperature variation within a normal range (i.e., 20ºC, 24ºC and 28ºC) affects: a) the overall impact of male-male competition on female lifetime reproductive success (i.e., male harm), b) how the net effects of harm are accomplished in terms of different female fitness components (i.e., reproductive rate,

actuarial ageing and reproductive ageing), and c) underlying male pre-copulatory (i.e., harassment) and post-copulatory (i.e., ejaculate toxicity) harm mechanisms (Fig. 1).

## MATERIALS AND METHODS

### Field Collection

In October 2018, we used banana traps to sample *Drosophila melanogaster* flies from three wineries in Requena (Spain): “Hispano-Suizas” (39.466128, -1.149642), “Pago de Tharsys” (39.497834, -1.122781) and “Dominio de la Vega” (39.515079, -1.143757). Traps were setup within the wineries, but in premises that were open to the exterior and to ample surrounding vineyards and/or in the vineyards themselves. After collection, we anaesthetized flies using mild $CO_2$ exposure. We then separated and individually distributed field-collected females in vials with standard food, left them to lay eggs for a period of 48 hours and then incubated their eggs at 24ºC, 60% humidity and a 12:12 dark-light cycle for 14 days to allow adult flies to emerge. We inspected the genital arch of F1 males of each of these female isolines to distinguish *D. melanogaster* isolines from *D. simulans*. We then collected three males and three females from each *D. melanogaster* isoline (total of 276 flies from 46 isofemale lines) and released them into a population cage with a surplus of food medium supplemented with live yeast, setting up the “Vegalibre (VG)” population. In November 2019 and October 2020, to maintain natural variation, we re-sampled the wineries and added 348 and 756 flies from 58 and 126 isofemale lines, respectively, following the same procedure (27 isofemale lines from “Pago de Tharsys” and 31 isofemale lines from “Dominio de la Vega” in November 2019 and 33 isofemale lines from “Pago de Tharsys”, 50 isofemale lines from “Dominio de la Vega” and 43 isofemale lines from “Hipano-Suizas” in October 2020).

### Stock maintenance and acclimation

We carried out all experiments between March 2020 and April 2021, using individuals from the VG field population kept in the laboratory with overlapping generations at an average temperature of 24°C with daily pre-programmed fluctuations (±4°C) mimicking natural daily temperature conditions during the reproductively active season, at ~60% humidity and on a 12:12-hr light: dark cycle (Pol Eko ST 1200 incubator). The lowest temperature was set up one hour after sunrise and the highest one hour after midday. It is important to note that our stock population of flies was kept under a programmed fluctuating temperature regime that

mimics their average circadian rhythm in the field, but temperature fluctuations in nature will be inherently subject to minor stochastic variations whose effects we controlled for (and thus did not capture) in this experiment. We used maize-malt medium (7g of agar, 72 g of malt, 72g of maize, 9g of soya, 15g of live yeast, 20g of molasses, and 33ml of Nipagin mix –3g of methyl 4-hydroxy-benzoate and 28ml of ethanol 90%– per 1000ml of water) as a food source throughout maintenance and experiments. To collect experimental flies, we introduced yeasted grape juice agar plates into stock populations to induce female oviposition. We then collected eggs and placed them in bottles containing ~75ml of medium to be incubated at 24 ± 4°C at a mean density of 223 ± 14.3 (95% CI) (Clancy & Kennington, 2001). We collected virgin flies within 6h of eclosion, under ice anaesthesia, and then we sexed and kept them in vials with food until their use (~3 days later), at 24 ± 4°C (see below for more details).

**Net impact of male harm on female fitness and underlying behavioural mechanisms (experiment 1)**

*Fitness assay*- To study whether male harm is affected by temperature, we established a factorial design to measure survival and lifetime reproduction success (LRS) of female flies under monogamy (i.e., one male and one female per vial) vs. polyandry (i.e., three males and one female per vial), across three stable temperature treatments typical of this population during their reproductively active period in the wild: 20, 24, and 28°C. Comparison of female fitness at monogamy vs. polyandry is standard procedure to gauge male harm in *Drosophila* and other organisms (see Yun, Agrawal, & Rundle, 2021 for a review and Gómez-Llano et al., 2023 for a recent meta-analysis). While the sex ratio in this species is typically 1:1, the operational sex ratio is male-biased and frequently reaches a 3:1 (or higher) male-bias in mating patches in the wild (Dukas, 2020). Thus, the 1:1 vs. 3:1 sex ratios used in this study represent biologically relevant scenarios and have actually become standard in *Drosophila* studies measuring male harm to females (Yun, Agrawal & Rundle 2021; Gomez-Llano et al. 2023).

We first collected virgin flies into same-sex vials of 15 individuals and then randomly allocated them to either of the three temperature treatments 48 hours before starting the experiment, at which temperatures they remained until its end. To estimate LRS, we transferred flies to fresh vials twice a week using mild $CO_2$ anaesthesia. We incubated the vials containing female eggs at 24 ± 4°C for 15-20 days (~15 days for vials coming from

28°C, ~17 days for 24°C and ~20 days for 20°C) to allow F1 offspring emergence, after which we froze them at -21°C for later counting. The differences in incubation time are due to differences in developmental time due to the temperature differences during the first 3-4 days of each vial (i.e., the time eggs remained at their respective temperature treatments before flipping females to new fresh vials and incubation at 24 ± 4°C). We discarded and replaced males with young (2-4 days old) virgin males (receiving the same treatment as described above for original males) three weeks after starting the experiment (at the same time for all treatments). In addition, we kept a stock of replacement males maintained at each of the three temperatures to replace dead male flies if needed. We kept focal female flies under these conditions for six weeks, after which we discarded males and followed females until they died for survival analysis (see Fig. S1.1 for an overview of the experimental design).

We started the experiment with 468 females (78 per temperature and mating treatment) and 936 males (234 per temperature in polyandry and 78 per temperature in monogamy). Due to discarded (e.g., accidentally damaged during handling) and escaped flies, final (female) sample sizes were: a) at 20°C, $n_{polyandry}$ = 74 and $n_{monogamy}$ = 76, b) at 24°C: $n_{polyandry}$ = 72 and $n_{monogamy}$ = 77, and c) at 28°C: $n_{polyandry}$ = 70 and $n_{monogamy}$ = 75. We estimated the overall degree of male harm by calculating the relative harm (H) following Yun et al. (2021):

$$H = \frac{W_{monogamy} - W_{polyandry}}{W_{monogamy}}$$

where *W* corresponds to female's fitness. Thus, H provides an estimate of the relative decrease in female fitness due to male harm.

Using the data collected above, we partitioned overall LRS effects into effects on early reproductive rate (i.e., offspring produced during the first two weeks of age), actuarial ageing (i.e., lifespan), and reproductive ageing (i.e., offspring produced over weeks 1-2 vs. 3-4). We used weeks 3-4 as an estimate of late reproductive rate because mortality was already evident at this point (i.e., reflecting ageing) and then was very high from week 5 onwards (Fig. S4.1; thus preventing accurate estimation of reproductive success).

Finally, we also calculated rate-sensitive fitness estimates for each individual female and treatment population. Rate-sensitive fitness estimates take into account when offspring are produced, not just how many offspring are produced, and thus allow estimating fitness

subject to population growth rate (Edward et al., 2011). This is important to understand how differences in the number and timing of offspring production translate into fitness under different demographic scenarios. For example, early reproduction is particularly favoured in increasing populations whereas late reproduction gains in importance in decreasing populations. Thus, while LRS is most suited to estimate individual fitness in stable populations, rate-sensitive estimates are preferred when $r \neq 0$ (Brommer, Merilä, & Kokko, 2002). We calculated both individual ($\omega_{ind}$) and population ($\omega_{pop}$) rate-sensitive fitness for the following intrinsic rates of population growth: r = -0.1, r = -0.05, r = 0, r = 0.05 and r = 0.1 (see Edward et al. 2011 for a detailed account). We then used $\omega_{pop}$ values to calculate the relative cost ($C_r$) of polyandry for each temperature treatment across different values of *r* as:

$$Cr = \frac{\omega_{pop\ (polyandry)}}{\omega_{pop(monogamy)}}$$

*Behavioural measures*- Immediately after the fitness experiment started, we conducted behavioural observations on the first day of the experiment across all treatments (Fig.S1.1). Our aim was to investigate the behavioural mechanisms that might underlie the potential fitness effects evaluated above. Due to logistic limitations, we conducted behavioural observations in the same temperature control room, so we had to conduct trials at 20, 24 and 28°C over three consecutive days (with both monogamy and polyandry treatments evaluated at the same time for each temperature), in a randomized order (i.e., 20, 28 and 24°C). Note that we collected virgin flies over three consecutive days to ensure all flies were 5 days-old at the start of the experiment. We recorded the following behaviours: (a) courtship intensity (number of courting males per female per hour), (b) male-male aggression rate (i.e., number of aggressions per hour) and (c) female rejection (i.e., number of rejections per hour; see Bastock & Manning, 1955; Connolly & Cook, 1973 for behavioural descriptions). We also recorded the number of total matings during the observation period.

Observations started at lights-on (10 a.m.) and lasted for 8 hours, during which time we continuously recorded reproductive behaviours using scan sampling of vials. Each complete scan lasted approximately 8 min, so that we always conducted one complete scan every 10 min to ensure recording all matings (see below). Scans consisted in observing all vials in succession for ca. 3s each and recording all occurrences of the behaviours listed above (i.e., all-occurrences recording of target behaviours combined with scan sampling). We interspersed behavioural scans with very quick (<1 min) mating scans where we rapidly

swept all vials for copulas at the beginning, in the middle and at the end of each complete scan. This strategy ensured that we recorded all successful matings (>10min), which typically last between 15 and 25min in our population of *D. melanogaster*. We obtained a total of 49 scans per vial. Behavioural observations were conducted only once, on day 1 of the fitness experiment, as prior experiments have shown that courtship, aggressive and female rejection behaviours in *D. melanogaster* are sufficiently stable over time so that our behavioural indexes are representative of long-term treatment differences (e.g., Carazo, Perry, Johnson, Pizzari, & Wigby, 2015; Carazo, Tan, Allen, Wigby, & Pizzari, 2014). In contrast to courtship, aggression and rejection indexes, note that total mating frequency over the first day cannot be taken as a reliable measure of mating rate (Wolfner, 1997). Thus, our rationale in recording this variable was just to ensure that early mating ensued normally across treatments (which was the case, see S5.2), but we did not include this variable in our statistical analyses.

**Mating effect on female reproduction and survival (experiments 2 to 5)**

To examine post-mating mechanisms that might underlie the fitness effects observed in our first experiment, we conducted four additional experiments to test whether temperature modulates the well-documented effects that mating with a male has on female receptivity, short-term fecundity and survival. In *D. melanogaster*, males manipulate female reproduction via their ejaculate, which increases male fitness but frequently decreases female lifespan and lifetime reproductive success (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995). Briefly, males transfer seminal fluid proteins (SFPs) produced by their accessory glands that stimulate female short-term fecundity, decrease female receptivity and ensure sperm storage, thus generally promoting male success in sperm competition (Chapman et al., 1995; Wigby & Chapman, 2005). In addition, prior studies have shown that males are able to tailor investment into SFPs according to perceived sperm competition risk (SCR) and intensity (Hopkins et al., 2019). Thus, we set-up a factorial design where we manipulated the temperature (i.e., 20, 24 and 28ºC) and perceived SCR levels (i.e., males kept alone vs. with 7 more males in a vial) at which adult focal males were kept prior to mating.

Then, we measured how reception of a treated male's ejaculate after a single mating in a common garden environment (i.e., all matings at 24ºC) affected female fecundity, survival and reproduction, following standard assays to gauge male ejaculate effects on females in

*D. melanogaster* (e.g., Chapman et al., 1995; Perry, Sirot, & Wigby, 2013; Wigby & Chapman, 2005; Wigby et al., 2009). We conducted separate experiments implementing two different temperature treatment durations (i.e., 48 hours and 13 days), to include two potential different scenarios. Our 48-hour treatment aimed to mimic short-term temperature effects on adult males whereas our 13-day treatment aimed to mimic longer-term effects on adult males that span a complete spermatogenesis cycle. The period from synthesis of deoxyribonucleic acid in the spermatocyte to successful insemination takes approximately 10d in *D. melanogaster* (Chandley & Bateman, 1962), so we treated males for 13d after sperm depleting them (see below) to ensure that males experienced treatment temperature across a whole spermatogenesis cycle.

*Receptivity assays (experiments 2 and 3)*- We first collected focal males as virgins (i.e., within 6h of eclosion) under ice anesthesia and randomly placed them either individually (low SCR) or in a same-sex group of 8 (high SCR) in plastic vials with food. Next, we randomly divided them into three groups that we allocated to the different stable temperature treatments for either 48 hours (i.e., short treatment duration, experiment 2, Fig. S1.2) or 13 days (i.e., long treatment duration, experiment 3, Fig. S1.3) immediately before the beginning of each experiment. For experiment 3, we depleted sperm and seminal fluid of focal males before allocating them to different temperature treatments by housing them with four standard virgin females for 24 hours, given that three successive matings are enough to deplete the accessory glands of male *D. melanogaster* (Linklater, Wertheim, Wigby, & Chapman, 2007; Macartney et al., 2021).

We collected all females and competitor males (i.e., standard males without any previous treatment) used in receptivity assays as virgins and held them in same sex groups of 15-20 flies at 24 ± 4°C. Experiments started by exposing all virgin females to single focal males for 2.5h at 24°C. After a successful copulation, we separated the mated females from the focal males and isolated them until the remating trial. We discarded unmated females and focal males. 72h after this first mating with the focal treated male, we individually exposed females to single virgin competitor males for 12h. After each trial, we transferred unmated females into a new vial with food, until the next remating trial on the next day (Fig. S1.2 & S1.3). We repeated remating trials for three consecutive days, which allowed us to calculate the cumulative percentage of remated females (and associated re-mating latencies; see below) for each of the three days of each experiment. Due to the large number of vials/flies involved, we conducted the experiments in two blocks each: with n = 390 females per batch in

experiment 2 (n = 436 rematings) and n = 420 females per batch in experiment 3 (n = 676 rematings). We also recorded mating duration for the first mating (i.e., with the focal treated male), the remating latency (i.e., time lapse between males being introduced into the female-containing vial and copulation) and mating duration for re-matings. Females and focal and competitor males were 4 days old for experiment 2. In experiment 3, females and competitor males were 4 days old, while focal males were 18 days old.

*Fecundity and survival assays (experiments 4 and 5)*- To study the effects of a single mating on female short-term fecundity and long-term survival, we performed two experiments (experiments 4 and 5, Fig. S1.4 & S1.5 respectively) where we compared female fecundity and F1 egg-to-adult viability of females mated with male flies subject to the same factorial design imposed in receptivity experiments (here experiment 4 had a treatment duration of 48 hours while experiment 5 had a treatment duration of 13 days). We collected and treated all focal males as in the receptivity assays described above, and then proceeded to mate virgin females in single pairs with focal males for 2.5h at 24°C. After copulation, we separated mated females from focal males and kept them individually in single vials. We discarded unmated females and focal males. We then transferred females to fresh vials every 24h for 4 days, and then every 3 days twice. Finally, we followed females until they died by combining them into same-treatment vials of 10 females that were flipped once a week. We removed dead flies at each flip and scored deaths on a daily basis. We counted eggs laid during the first 3 days and incubated vials from days 1, 2, 3, 4, 5, and 8 until adults emerged to count progeny and determine egg-to-adult viability (Fig. S1.4 & S1.5). Sample sizes were of 545 females for experiment 4, and 480 females for experiment 5. Females and focal or competitor males were 4 days old for experiment 4. In experiment 5, females and competitor males were 4 days old, while focal males were 18 days old.

**Statistical analyses**

We performed all statistical analyses using R statistical software (version 3.5.2). In all models, we assessed fit and validated all models by visual inspections of diagnostic plots on raw and residual data (Zuur, Ieno, & Elphick, 2010). In all models, we used ANOVA type III test "F" to compute *p*-values corrected by the Benjamini-Hochberg (BH) method to control the inflation of type I error-rate due to multiple testing. We fitted all models with temperature effect as a covariate, given that it is a continuous variable. In all cases where we detected a significant interaction between main effects, we ran models separately for each temperature

(or treatment duration for experiments 2 to 5) to explore the nature of such interactions. As a complementary analysis, in these cases we re-fitted the original model with temperature as factor, which allowed us to run a post hoc Tukey's test as an additional way to explore interactions while controlling for inflation of experiment-wise type 1 error rate. We provide the latter in the SM, but we note that both approaches always yielded qualitative identical results.

*Experiment 1*- To examine temperature effects on male harm, we evaluated the interaction between mating system and temperature on female fitness (LRS), early reproductive rate, reproductive ageing, actuarial ageing and male and female reproductive behaviours (courtship intensity and female rejection; experiment 1). We fitted generalized linear models (GLMs) with temperature, mating system and their interaction as fixed effects. Graphical inspection of LRS, actuarial ageing, and reproductive behaviours (courtship intensity and male-male aggression behaviours) revealed that the normality assumption was apparently violated, as well as the independence assumption for LRS. Box–Cox transformation (Quinn & Keough, 2002) solved these problems and allowed us to run a GLM with a Gaussian error distribution. We compared GLMs with their corresponding null GLMs using likelihood ratio test only to test the significance of the independent variables in the full model. We detected collinearity between mating system and the interaction in LRS, early reproductive, reproductive ageing, actuarial ageing, courtship intensity and female rejection models. In all these cases, we thus refitted the model without the main mating system effect (which was not our main interest). As a complementary analysis for LRS, we also ran a model with temperature as factor and a predetermined quadratic contrast table (given the relationship between LRS and temperature is clearly non-linear, Fig. 2), and obtained similar results.

We also used Cox proportional hazards survival model to analyse potential differences in mortality risk across treatments, using the *survival* and *survminer* packages (Therneau, 2015; Kassambara & Kosinski, 2018), which allowed us to include the females lost during manipulations as "right censored" individuals (i.e., individuals that are taken into account for demographic analysis until the day they disappear, Kleinbaum & Klein, 2012). We analysed female rejection behaviours in two different ways. First, we examined mating system and temperature effects on overall rejection rates using a Gamma distribution, as this variable was continuous and zero-inflated. Second, we also used a binomial GLM to estimate potential differences in female rejections per courtship. Finally, we analysed male-male

aggression with mating system as the sole main factor (as male-male aggressions where only possible in the polyandry treatment).

*Experiments 2-5*- To examine the effect of temperature on post-copulatory effects, we evaluated the effect of SCR, temperature, treatment duration and their interaction on receptivity (mating duration and remating latency -experiments 2 and 3-) and fecundity -oviposition and egg viability- and female survival (experiments 4 and 5). For mating duration, remating latency and egg viability we fitted generalized linear models (GLMs) with temperature, SCR level, treatment duration and their interaction as fixed effects. We assessed significance of factors by dropping individual terms from the full model using the "drop1" function, refitting models without the triple interaction where necessary. We detected a problem of collinearity between SCR and the interactions, as well as between treatment duration and the interactions in mating duration, remating latency and egg viability models. In all these cases, we refitted the model without the main SCR and treatment duration effects (which were not our main interest). For mating duration, we used a Gamma distribution. For remating latency and egg fertility, we used a Gaussian distribution. For oviposition, we fitted a generalized linear mixed model (GLMM) with temperature, SCR level, treatment duration and their interaction as fixed effects and day as a random effect. Initially, we run a model with a zero inflated distribution, in which the zero values are modelled separately from the non-zero values (Zuur et al., 2010). However, we detected problems of collinearity, including treatment duration as an effect. We thus run two separate models for each treatment duration using Hurdle models without the main SCR effect. Finally, for survival we used a Cox proportional hazards survival model to analyse potential differences in mortality risk across treatments, including the females lost during manipulations as "right censored" individuals.

## RESULTS

<u>Net impact of male harm on female fitness and underlying behavioural mechanisms (experiment 1)</u>

*LRS*- We detected a significant temperature by mating system interaction for female lifetime reproductive success ($F_{1,425}$= 16.931, $p$ < 0.001, Fig. 2 & S2.1), and a marginally non-significant temperature effect ($F_{1,425}$= 3.712; $p$= 0.054). Separate models for each temperature level show a larger effect of mating system on lifetime reproductive success at 24°C than at 20°, and larger at 20° than at 28°C, despite 95 % CI of the estimates overlap

(Tables 1 & S1.1a). The decrease in LRS in polyandry vs. monogamy peaked at 24°C (H = 0.36) and was 1.6 (H = 0.22) and 3.4 times (H = 0.10) smaller at 20 and 28°C, respectively. Rate-sensitive fitness estimates show that estimated population costs are dependent on background growth rates (Fig. 3), and in general particularly accused in decreasing populations.

*Early reproductive rate*- We did not detect a significant temperature by mating system interaction ($F_{1,425}$= 2.94; $p$= 0.09). We did detect a significant main temperature effect ($F_{1,425}$= 64.63; $p$ < 0.001; Fig. S2.2), such that early reproduction increased at 28ºC.

*Reproductive ageing*- We detected a significant temperature by mating system interaction for reproductive ageing ($F_{1,425}$= 55.24; $p$ < 0.0001; Fig. S2.2), and a clear main effect for temperature ($F_{1,425}$= 44.25; $p$ < 0.001). Running models separately for each temperature level showed that mating system affected reproductive ageing at all temperatures, but particularly so at 24ºC and 28ºC (Table 1 & S1.1a, Fig. S2.2).

*Actuarial ageing*- We detected a significant temperature by mating system effect for lifespan ($F_{1,428}$= 73.81; $p$ < 0.0001; Fig. 4 & S4.1a), and a significant main effect for temperature ($F_{1,428}$= 36.98; $p$ < 0.001). Mating system affected actuarial ageing at all temperatures, but particularly so at 20ºC (Tables 1 & S1.1a). The survival analysis yielded qualitatively identical results (Table S1.2, survival plot Fig. S4.1b).

**Table 1**. Output from separate GLMs for each temperature level to explore significant interactions between temperature and mating system effects on female fitness components.

| ***T*°C** | *LRS* | | | *Reproductive ageing* | | | *Actuarial ageing* | | |
|---|---|---|---|---|---|---|---|---|---|
| | $F_{df}$ | *p value* | *Estimate (95%CI)* | $F_{df}$ | *p value* | *Estimate (95%CI)* | $F_{df}$ | *p value* | *Estimate (95%CI)* |
| 20° | $4.4_{1,145}$ | **0.039** | 1.07 (0.06- 2.06) | $12.1_{1,145}$ | **<0.001** | -7.99 (-12.5- -3.5) | $39.6_{1,148}$ | **<0.001** | 7.44 (5.1 - 9.8) |
| 24° | $16.6_{1,142}$ | **<0.001** | 22.39 (11.6- 33.1) | $35.3_{1.142}$ | **<0.001** | -17.2 (-22.9- -11.5) | $32.2_{1,143}$ | **<0.001** | 4.84 (3.2 - 6.5) |
| 28° | $2.2_{1,135}$ | 0.137 | 1.88 (-0.58- 4.36) | $14.1_{1,135}$ | **<0.001** | -11.87 (-18.1- -5.7) | $19.7_{1,137}$ | **<0.001** | 2.97 (1.7 - 4.3) |

## Reproductive behaviour

The interaction between temperature and mating system was significant for courtship rate ($F_{1,441}$= 45.62; $p$ < 0.0001), and we also detected a main temperature effect ($F_{1,441}$= 16.69; $p$ < 0.0001 Fig. 5a & S5.1a). Running models separately for each temperature level, mating system affected courtship rate at 24° and 28° but not at 20°C (Tables 2 & S1.1b). Likewise, we detected a significant temperature by mating system effect for rejection rate ($F_{1,441}$= 24.48; $p$ < 0.0001 Fig. 5b & S5.1b), and a main effect for temperature ($F_{1,441}$= 5.61; $p$ = 0.020). Models for each temperature level show a mating system effect at 24 and 28°C but not at 20°C (Tables 2 & S1.1b). We did not detect a significant interaction between temperature and mating system ($F_{1,294}$= 0.30; $p$ = 0.582), nor a temperature effect ($F_{1,294}$= 0.08; $p$ = 0.773), for rejection rates per courtship. For male-male aggression rate we detected a significant temperature effect ($F_{1,214}$= 14.45; $p$ = 0.0003 Fig. 5c & S5.1c).

**Table 2**. Output from separate GLMs for each temperature level to explore significant interactions between temperature and mating system effects on underlying behaviorual mechanisms. p-values were corrected for multiple testing using Benjamini-Hochberg correction.

| ***T*°C** | *Courtship rate* | | | *Rejection rate* | | |
|---|---|---|---|---|---|---|
| | $F_{df}$ | *p value* | *Estimate (95%CI)* | $F_{df}$ | *p value* | *Estimate (95%CI)* |
| 20° | $0.4_{1,148}$ | 0.546 | -0.04 (-0.16-0.08) | $0.20_{1,148}$ | 0.654 | -0.05 (-0.30- 0.19) |
| 24° | $21.8_{1,147}$ | **<0.001** | -0.40 (-0.57- -0.23) | $10.9_{1.147}$ | **0.001** | -17.2 (-1.01- -0.25) |
| 28° | $40.2_{1,143}$ | **<0.001** | -0.63 (-0.83- -0.43) | $19.3_{1,143}$ | **<0.001** | -11.87 (-0.96- -0.36) |

## Mating effects on female reproduction and survival (experiments 2 to 5)

*Female receptivity (experiments 2 and 3)*- For the duration of the first mating in our female receptivity assays, we detected significant SCR by temperature ($F_{1,1239}$= 40.42; $p$ < 0.0001), treatment duration by temperature ($F_{1,1239}$= 5.97; $p$ = 0.024), and SCR by treatment duration ($F_{1,1239}$= 5.48; $p$ = 0.024) interactions (Fig. 6a & S6.1a). We found no significant main effect for temperature ($F_{1,1239}$= 3.36; $p$ = 0.07). Running models separately for each temperature,

SCR affected the duration of the first mating at 20, 24 and 28°C, while treatment duration only affected the duration of the first mating at 24°C (Table 3a & S3.1a-b). Additionally, running models for each treatment duration, SCR affected the duration of the first mating at short and long treatment durations (Table 3b & S3.1c). Similarly, for female remating latency, we detected significant interactions for SCR by temperature ($F_{1,1094}$= 6.15; $p$ = 0.022), and treatment duration by temperature ($F_{1,1094}$= 5.17; $p$ = 0.028), whereas the interaction between SCR by treatment duration was not significant ($F_{1,1094}$= 1.00; $p$ = 0.316) (Fig. 6b & S6.1b). We also detected a significant main temperature effect ($F_{1,1094}$= 8.21; $p$ = 0.01). Running models separately for each temperature level, SCR level and treatment duration only affected remating latency at 28°C (Table 3a & S3.1a-b).

**Table 3**. Model outputs from separate GLMs for each (a) temperature level and (b) treatment duration to explore significant interactions.

a)

| ***T*°C** | ***Effect*** | ***Mating duration*** | | | ***Remating latency*** | | |
|---|---|---|---|---|---|---|---|
| | | *$F_{df}$* | *p value* | *Estimate (95%CI)* | *$F_{df}$* | *p value* | *Estimate (95%CI)* |
| 20° | *Sperm competition risk* | $3.9_{1,423}$ | **0.046** | 0.03 (0.0005 - 0.05) | $0.95_{1,377}$ | 0.330 | 27.9 (-28.2 - 84.2) |
| | *Treatment duration* | $2.3_{1,423}$ | 0.133 | -0.02 (-0.04 - 0.006) | $0.0006_{1,377}$ | 0.980 | -0.73 (-58.5 - 57.0) |
| 24° | *Sperm competition risk* | $10.6_{1,405}$ | **0.001** | 0.05 (0.02 - 0.07) | $0.07_{1,358}$ | 0.779 | -8.47 (-67.7 - 50.7) |
| | *Treatment duration* | $3.7_{1,405}$ | **0.054** | -0.02 (-0.05 - 0.0003) | $0.04_{1,358}$ | 0.842 | -6.24 (-67.8 - 55.3) |
| 28° | *Sperm competition risk* | $26.5_{1,410}$ | **<0.001** | 0.084 (0.052 - 0.117) | $8.05_{1,358}$ | **0.005** | 87.81 (27.1 - 148.4) |
| | *Treatment duration* | $0.6_{1,410}$ | 0.451 | -0.12 (-0.04 - -0.2) | $9.73_{1,358}$ | **0.002** | -97.65 (-158.9 - -36.3) |

b)

| ***Treatment duration*** | ***Mating duration*** | | |
|---|---|---|---|
| | *$F_{df}$* | *p value* | *Estimate (95%CI)* |
| Short (48 hours) | $4.5_{1,554}$ | **0.033** | 0.03 (0.002 - 0.06) |
| Long (13 days) | $54.2_{1,686}$ | **<0.001** | 0.07 (0.051 - 0.089) |

*Female fecundity and survival (experiments 4 and 5)*- For the number of eggs produced by females during the three first days, we did not detect significant interactions between temperature and SCR for either short or long treatment durations, nor a main significant effect for temperature (Fig. S6.2 & table S4.1): i) Short treatment duration, SCR by temperature interaction ($\chi^2_1$= 0.05; $p$= 0.82), temperature effect ($\chi^2_1$= 2.82; $p$= 0.09), ii) Long treatment duration, SCR by temperature interaction ($\chi^2_1$= 0.03; $p$= 0.84), temperature effect ($\chi^2_1$= 0.37; $p$= 0.54). Likewise, for the total number of offspring produced by females during days 1, 2, 3, 4, 5, and 8 after mating, we did not find significant interactions between SCR and treatment duration ($F_{1,952}$= 0.022; $p$ = 0.881), or between SCR and temperature ($F_{1,952}$= 0.418; $p$ = 0.674), but we did between temperature and treatment duration ($F_{1,952}$= 9.599; $p$ = 0.005) (Fig. S6.3). We did not detect a main temperature effect ($F_{1,952}$= 2.797; $p$ = 0.157). Running models for each temperature level, we found that treatment duration affected the total number of offspring produced by females at 24 and 28°C but not at 20°C (Tables 4 & S4.2).

**Table 4** Summary statistics from fitting GLMs separately for each temperature level to explore the significant interaction between temperature and treatment duration effects for total of offspring produced by females during the days 1, 2, 3, 4, 5, and 8 after mating.

| ***T*°C** | *Total of offspring* | | |
|---|---|---|---|
| | $F_{df}$ | *p value* | *Estimate (95%CI)* |
| 20° | $0.6_{1,322}$ | 0.454 | 1.42 (-2.30 – 5.15) |
| 24° | $4.6_{1,321}$ | **0.032** | 4.11 (0.35 – 7.86) |
| 28° | $5.2_{1,308}$ | **0.022** | 4.26 (0.62 – 7.89) |

Finally, we did not detect significant interactions in survival (SCR by treatment duration, $\chi^2_1$= 0.276, $p$ = 0.694; SCR by temperature, $\chi^2_1$= 0.311, $p$ = 0.694; temperature by treatment duration, $\chi^2_1$= 4.09, $p$ = 0.128), nor significant main temperature ($\chi^2_1$=1.69; $p$ = 0.386) or SCR effects ($\chi^2_1$= 0.154; $p$ = 0.694). We did detect a significant main treatment duration effect ($\chi^2_1$= 13.03; $p$ = 0.001; Fig. S6.4).

## DISCUSSION

We show that male harm exhibits complex plasticity in response to temperature changes within an optimal reproductive range (20ºC-28ºC) for a wild *D. melanogaster* population, with

several implications for our understanding of how sexual conflict unfolds in nature. First, we show that net harm to females varies markedly within this range, such that relative harm (i.e., the proportional reduction in average female LRS in polygamy vs. monogamy) was maximal at 24ºC, decreased at 20ºC and was minimum at 28ºC. Second, rate-sensitive fitness estimates indicate that arising population costs are dependent on the interaction between temperature and population demography, such that demography modulates the impact of male harm on population viability less at warmer temperatures. Third, our results strongly suggest that male harm effects on population growth have to do with the fact that different mechanisms exhibit qualitatively different reaction norms in response to temperature, with distinct effects on different female fitness components. More specifically, at cold temperatures courtship intensity (i.e., male harassment; pre-copulatory harm mechanism) decreased and female fitness was impacted more via accelerated actuarial ageing than at warm temperatures. In contrast, warm temperatures impacted mating costs and effects on female receptivity (i.e., post-copulatory mechanism), and female fitness decreased more via accelerated reproductive ageing than at colder temperatures. We discuss how such plasticity may affect how male harm impacts populations, as well as selection, adaptation and, ultimately, evolutionary rescue under a warming climate.

*Temperature effects on male harm and its consequences for populations*

We found that temperature variation within the optimal reproductive range for our study population in the wild had a strong effect on net male harm levels. To gauge male harm, we used the standard procedure of comparing female LRS in monogamy, which imposes low male-male competition and thus low sexual conflict, vs. polyandry (i.e., a female with three males), which imposes high male-male competition and intensifies sexual conflict between the sexes (Yun et al., 2021). These sex ratios are common in mating patches in the wild, and are actually representative of the extremes in natural levels of male-male competition (Dukas, 2020). In monogamy, temperature did not affect female fitness (Fig. 2), showing that female reproduction is indeed optimal within this range. In contrast, the net decrease in female LRS in polyandry vs. monogamy was highly dependent on the thermal environment, with an average decrease of H = 0.36 at 24ºC, H = 0.22 at 20ºC and H = 0.10 at 28ºC, at which temperature we did not find a statistically significant effect of mating system on female LRS (Fig. 2).

Male harm effects are expected to be cumulative over time, so that their impact on female survival and reproductive output is unlikely to be constant across a female's lifespan (Bonduriansky et al., 2008; Filice et al., 2020). At the same time, early vs. late life reproduction weigh differently on both individual fitness and how this impacts background population growth, depending on whether such population is decreasing, stable or growing (Edward et al., 2011; Priest et al., 2008). Thus, in order to evaluate how male harm is likely to impact populations across different temperatures, we calculated rate-sensitive fitness estimates for individual (Fig. 3a) and population (Fig. 3b) fitness across a range of demographic scenarios (i.e., decreasing, stable and growing populations; Edward et al., 2011). Overall, the impact of male harm was higher in decreasing populations, where late-life reproduction gains importance, which is consistent with the idea that male harm effects are cumulative over the lifespan of females. Above and beyond this general effect, the observed interaction between temperature and female fitness is maintained irrespective of population demography. That is, male harm decreases female individual fitness more at 24ºC than at 20ºC and 28ºC. Interestingly, though, temperature also has a clear effect on how demography affects population-level costs. At hotter temperatures the relative population costs of male harm vary considerably less with demography (i.e., background population growth) than at colder temperatures (Fig. 3b). For a decreasing population, the relative population costs of male harm are significantly higher at 20ºC than at 28ºC, but this difference wanes as population growth rate (r) increases, to the point of reverting in a rapidly growing population (Fig. 3b). This suggests that male harm has more impact on late-life female fitness at cold temperatures, and hints at the possibility that cold vs. hot temperature affect qualitatively distinct parameters of female fitness, and thus underlying mechanisms of male harm.

Looking at effects on separate female fitness components yields results largely in agreement with the above ideas. Again, consistent with the fact that harm needs to accumulate in time to impact female fitness, temperature had no effect on how or whether male competition impacted early reproductive rate (Fig. S2.2). Temperature did, however, modulate how male harm impacted on female actuarial vs. reproductive ageing. We found clear differences in female lifespan across temperature and mating system treatments. Male harm effects on actuarial ageing (an increase in mortality rate with age) were more severe at 20°C (35% decrease in female lifespan) than at 24°C (31% decrease) and at 28°C (22% decrease; Fig. 3). In contrast, while male harm accelerated reproductive ageing at all temperatures, this

decrease was more marked at 24°C and at 28°C than at 20°C (Table 1; Fig. S2.2). In accordance with available evidence in lab-adapted flies (García-Roa et al., 2019), these results show that temperature does not seem to have a linear effect on ageing processes.

To sum up, we offer strong evidence that different male harm mechanisms are sensitive to temperature in different ways, with ensuing modulation of its effects on different female fitness components. We underscore two potential consequences arising from these findings. The first is that the net fitness effects of male harm might be lower than expected when considered in its natural thermal setting, because previous research has focused on studying male harm at average temperatures, precisely where we found it to be maximal. It follows that integrating harm across the natural temperature range will result in a lower net decrease in female fitness in the wild. The second is that environmental variability may foster the maintenance of genetic variation underlying different mechanisms of male harm, and even potentially divergent male-male competition strategies. Our work joins an increasing number of recent studies in highlighting the importance of evaluating more ecologically realistic scenarios in sexual conflict research, particularly how natural fluctuations in the ecology of the socio-sexual context may affect sexual conflict processes (García-Roa et al., 2019, 2020; Gomez-Llano et al., 2018; Yun et al., 2017).

In addition, the above results open up the possibility that warm climates may lessen the impact of sexual conflict on population viability, perhaps facilitating evolutionary rescue. Male harm effects were found to be relatively lower in warmer temperatures and in decreasing populations, precisely the type of context that would be typical of a climate-change scenario. The effects found in this study where within the optimum reproductive range for this population, but similar results have been reported in response to stressful temperatures. For instance, temperature has been used to induce environmental stress in natural populations of seed beetles (*Callosobruchus maculatus*), showing that a stressful thermal regime reduces intra-locus sexual conflict by aligning selection in males and females (Berger et al., 2014, but see Martinossi-Allibert et al., 2019). A previous study in a lab-adapted population of *D. melanogaster* also shows that male harm levels (i.e. inter-sexual conflict) decrease when subject to maladaptive warm temperatures (García-Roa et al., 2019). However, there are two reasons why it is important to caution against direct extrapolation of our results to wild populations at this stage, in particular in relation to their relevance for populations facing the current climate crisis. First, our monogamy vs. polyandry treatments reflect the low vs. high-end of the spectrum of operational sex ratios

that are typical of *D. melanogaster* at mating patches in the wild (Dukas 2020), and thus our measures of male harm effects are likely to be generally higher than expected in nature. While this does not change the main conclusions regarding temperature effects, it is important to note when considering the degree to which these effects may be relevant in the wild. Second, our treatment temperatures were stable, designed to study how coarse-grain changes in temperature across the adult lifespan of flies may influence how sexual conflict unfolds in nature. Thus, future studies will need to encompass how fine-grained fluctuation (i.e., repeated variation of temperature across an adult's lifespan) may affect male harm for a more comprehensive picture of temperature effects on sexual conflict in the wild.

*Temperature effects on sexual conflict mechanisms in Drosophila melanogaster*

Prior to mating, *D. melanogaster* males harm females via sexual harassment, due to protracted courtship of one or several males that results in physical damage, interference with other behaviours (e.g., foraging or egg-laying) and costly energetic investment into male avoidance (e.g., female rejection) (Bretman & Fricke, 2019; Partridge & Fowler, 1990; Teseo et al., 2016). Importantly, previous studies in this species have shown that male harm is directly related to courtship intensity and female rejection, and indirectly to male-male aggression as a direct measure of male intrasexual competition (e.g., Bretman & Fricke, 2019; Carazo et al., 2014; Partridge & Fowler, 1990). In our study, we found a clear increase in both courtship intensity and female rejection in polyandry vs. monogamy, but this was largely dependent on the thermal environment (Fig. 5a-b). While we found clear evidence that harassment increases in polygamy at 28°C and 24°C, we did not find a similar effect at 20°C (Fig. 5a). Female rejection behaviours exhibited the exact same trend (Fig. 5b), and this was due to increased male courtship attempts (not to an increase in female likelihood to reject male courtships). Thus, our results suggest that male harassment decreases drastically at cold temperatures and is perhaps maximal at warm temperatures, at which temperatures we also detected the highest level of male-male aggression (Fig. 5c). The above results seem to suggest that the decrease in male harm to females that we detected at 20ºC vs. 24ºC (Fig. 2) is likely explained by the substantial drop in male harassment at this temperature. However, the same logic cannot apply for the decrease in male harm to females that we detected at 28°C vs. 24°C (see below).

During mating, *D. melanogaster* males transfer seminal fluid proteins (SFPs) that manipulate female re-mating and egg-laying rates to their advantage, but this normally comes at a cost

to females in terms of lifespan and lifetime reproductive success (Chapman, Bangham, et al., 2003b; Chapman et al., 1995; Sirot et al., 2009; Wigby & Chapman, 2005). Furthermore, these effects are known to be modulated by the socio-sexual context, such that males strategically adjust their investment in SFPs depending on expected SCR levels (Bretman, Fricke, & Chapman, 2009; Hopkins et al., 2019; Wigby et al., 2009). We run a series of standard assays in *Drosophila* (Bretman et al., 2009; Hopkins et al., 2019; Wigby et al., 2009) to investigate whether temperature modulates any of the known phenotypic effects that SFPs have on females following a single mating with males that were exposed to low (i.e., kept alone in their vial) or high SCR (i.e., kept with 7 other males in a vial). The temperature males were kept at prior to mating did not modulate how mating affected short-term female fecundity or lifespan, but we did detect a clear effect of temperature with respect to female receptivity. Generally, reception of a male ejaculate resulted in a sharper decrease in female receptivity (i.e., longer remating latency) when males perceived a high SCR, which is to be expected and is in accordance with previous studies (Bretman et al., 2009; Bretman, Fricke, Hetherington, Stone, & Chapman, 2010; Denis et al., 2017). However, this effect was largely modulated by temperature, such that it was more clearly detected at 28ºC (Fig. 6b). Interestingly, this effect was consistent for males treated for 48h and 13 days (albeit more marked in the latter case), and these results were paralleled by temperature effects on mating duration (Fig. 6a). Namely, males exposed to high SCR generally mated for longer than males exposed to low SCR, again in line with previous findings (Bretman et al., 2010; Bretman, Westmancoat, Gage, & Chapman, 2013), but this difference was clearly larger at 28ºC (Fig. 6a). It is worth restating that, under monogamous conditions, we did not detect a decrease in female productivity at 28ºC vs 24ºC and 20ºC, so that general differences in sperm viability are unlikely to account for the aforementioned differences. A potential explanation for these results is that males may perceive a higher SCR when kept in groups at warmer temperatures (e.g., due to increased activity). This, however, would predict the same differences between 24ºC and 20ºC, which was not the case.

All in all, our results suggest that at least some post-copulatory harm mechanisms are sensitive to temperature, because receipt of a male ejaculate resulted in a sharper decrease of female receptivity in high vs. low SCR at warm temperatures (particularly after 13d of exposure). We speculate that this may contribute to explain why male harm drops so sharply at 28ºC despite the fact that male harassment and male-male competition seems to be maximal at this temperature. In *D. melanogaster*, as in many other species, repeatedly

mating is costly for females in terms of lifetime reproductive success (Arnqvist & Nilsson, 2000; Chapman et al., 1995; Fowler & Partridge, 1989). An intriguing possibility is thus that either SFPs are more effective at lowering female re-mating rates or males are investing relatively more in SFPs with increasing SCR at warm temperatures, thereby buffering these costs. An alternative (but complementary) possibility is that temperature may affect female behaviour or physiology in a way that makes them more resistant to harm.

We suggest future studies should explore these ideas by examining in detail how temperature affects the composition and transfer of SFPs to females, and how females respond to the transfer of these proteins and to male harm in general (i.e. effects on female resistance). In combination with experimental evolution at different temperatures, such an approach would allow us to disentangle between two causal hypotheses for the observed results. First, that warm temperatures may buffer sexual conflict in itself by aligning male and female reproductive interests. For example, if live-fast-die-young strategies fare relatively better for females at warm than cold temperatures, male and female optimal reproductive strategies may overlap more due to the fact that cumulative late-life effects of male harm might be diluted by the inherently high female intrinsic mortality at warm temperatures. Second, whether modulation of male harm at cold and (particularly) warm temperatures has to do with the fact that different male harm mechanisms are adapted to operate better at certain temperatures. For example, due to environmental effects on male activity or protein folding. In the latter case, male harm would be expected to increase as males adapt to higher or lower average temperatures, but sexual conflict *per se* (i.e. the degree to which male and female evolutionary interests overlap) would be expected to remain constant. Both of the above hypotheses could have broad consequences for our understanding of the evolution of sexual conflict across the tree of life.

*Conclusions*

Our findings may have implications for our understanding of how sexual conflict unfolds in nature, and its consequences for populations. First, they add to growing evidence (Gomez-Llano et al., 2018; MacPherson et al., 2018; Malek & Long, 2019; Perry & Rowe, 2018; Yun et al., 2017) indicating that ecological context is key in shaping sexually antagonistic coevolution and, in particular, suggest that temperature may be a particularly salient ecological factor to understand how sexual conflict evolves and operates in nature (García-Roa et al., 2020). Second, they highlight that male harm mechanisms can be highly plastic

even in response to relatively minor fluctuations in temperature well within the optimal reproductive range, and suggest that different harm mechanisms are differently affected by temperature. Third, they suggest that male harm effects on female life-history and fitness components are asymmetrically modulated by temperature; male harm particularly decreased survival at cold and moderate temperatures, and reproductive ageing at moderate and hot temperatures. In conjunction, these phenomena may have a bearing on evolutionary rescue and local adaptation processes. For example, in maintaining genetic variation in sexually selected traits in males, and/or in ameliorating the demographic impact of sexual conflict in populations facing environmental change.

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## COMPETING INTERESTS

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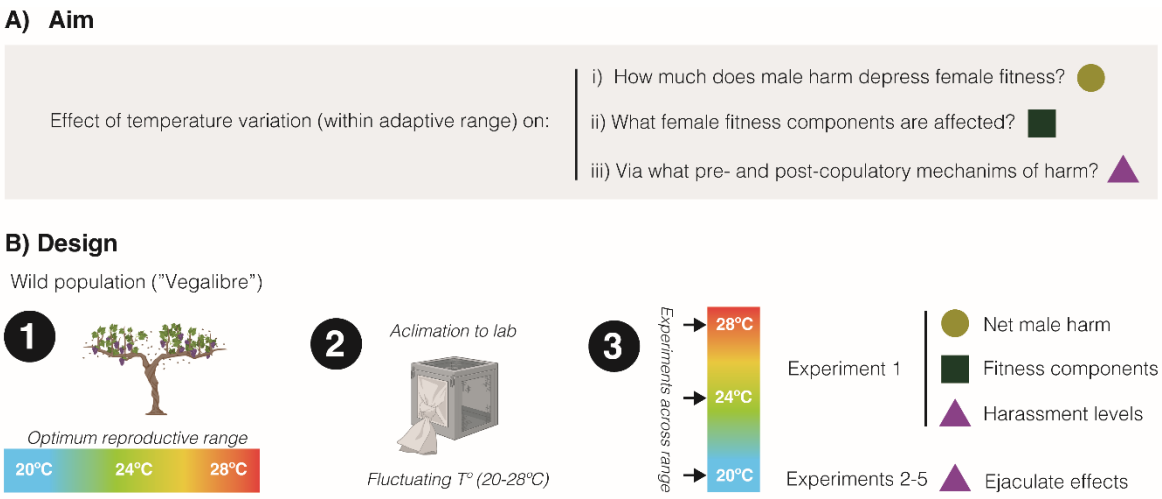



**Figure 1**. Schematic overview of study. A) Our aim was to study how temperature variation, across a range at which reproduction is optimum in the wild, may affect: the net decrease in female fitness resulting from male harm, what female fitness components are mainly affected by male harm, and what pre-copulatory (i.e., sexual harassment) and post-copulatory (i.e., ejaculate effects on female receptivity, short-term fecundity and survival) mechanism of harm. B) General design of study: 1) We sampled a wild population of Drosophila melanogaster flies that reproduce optimally between 20ºC and 28ºC, 2) We setup a population in the lab and left it to acclimate for a few generations under a programmed fluctuating temperature regime that mimics wild conditions in late spring-early summer (20ºC-28ºC range with mean at 24ºC), 3) We run a series of 5 experiments (each repeated at 20ºC, 24ºC and 28ºC) to study temperature effects on net male harm, female fitness components and male pre- and post-copulatory mechanisms of harm.

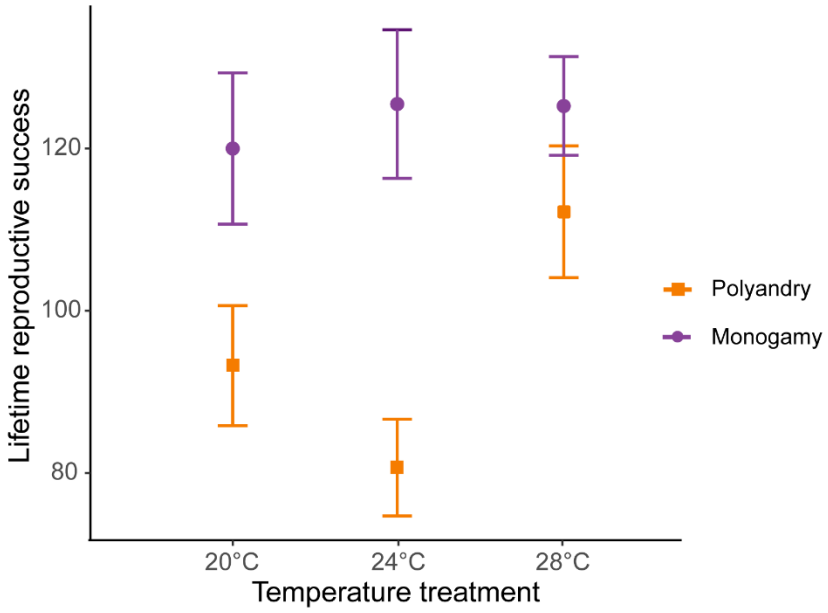



**Figure 2**. Female lifetime reproductive success (mean ± SEM) across temperature and mating system treatments.

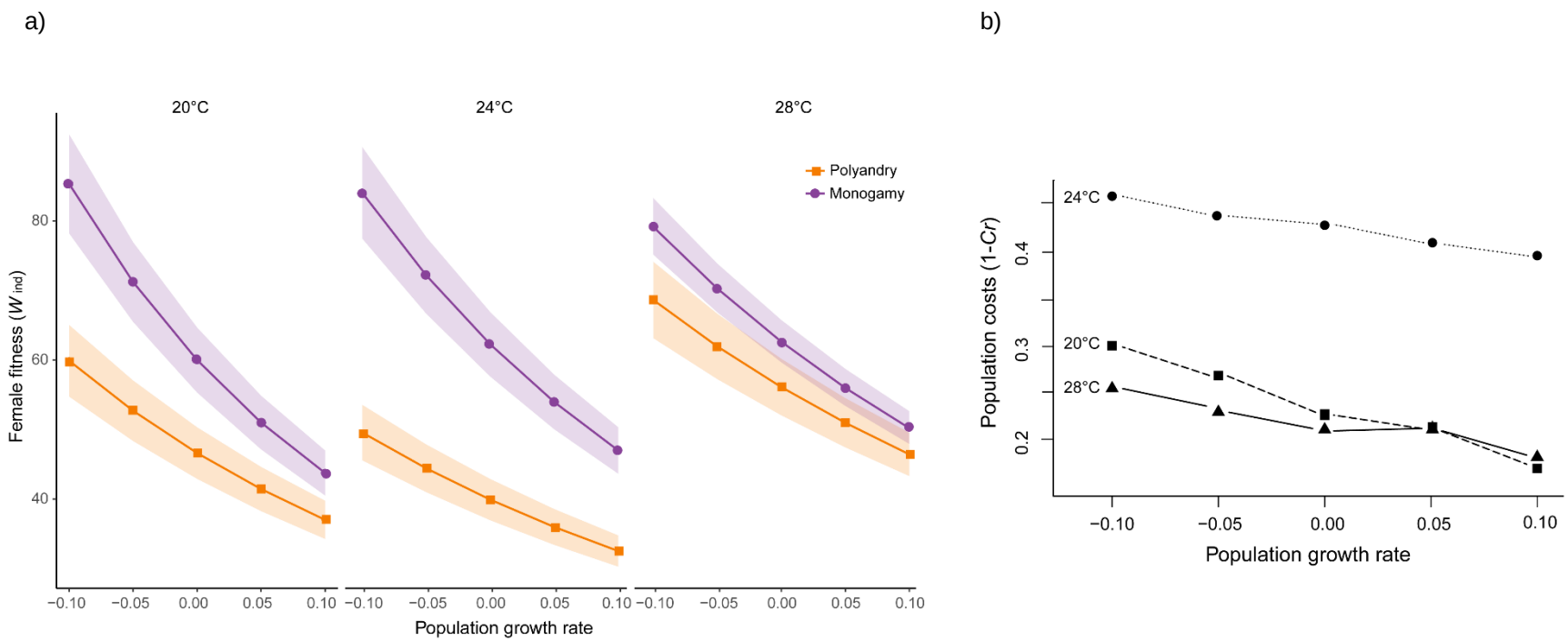



**Figure 3.** a) Average rate-sensitive index fitness estimate of individual females (Mean $\omega_{ind}$) for different population growth rates across temperature and mating system treatments (shaded areas denote SEM). b) Relative cost ($C_r$) of polyandry (vs. monogamy) for each temperature treatment for different population growth rates. *Cr* was calculated based on rate-sensitive index fitness estimates for populations ($\omega_{pop}$), whereby population costs are shown as 1 – *Cr,* thus reflecting the relative decrease in population growth rate.

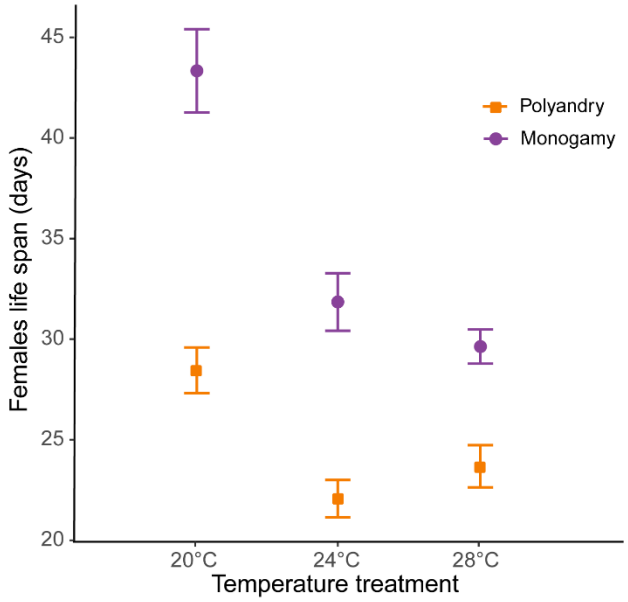



**Figure 4.** Male harm effect on female lifespan (mean ± SEM) across temperature and mating system treatments.

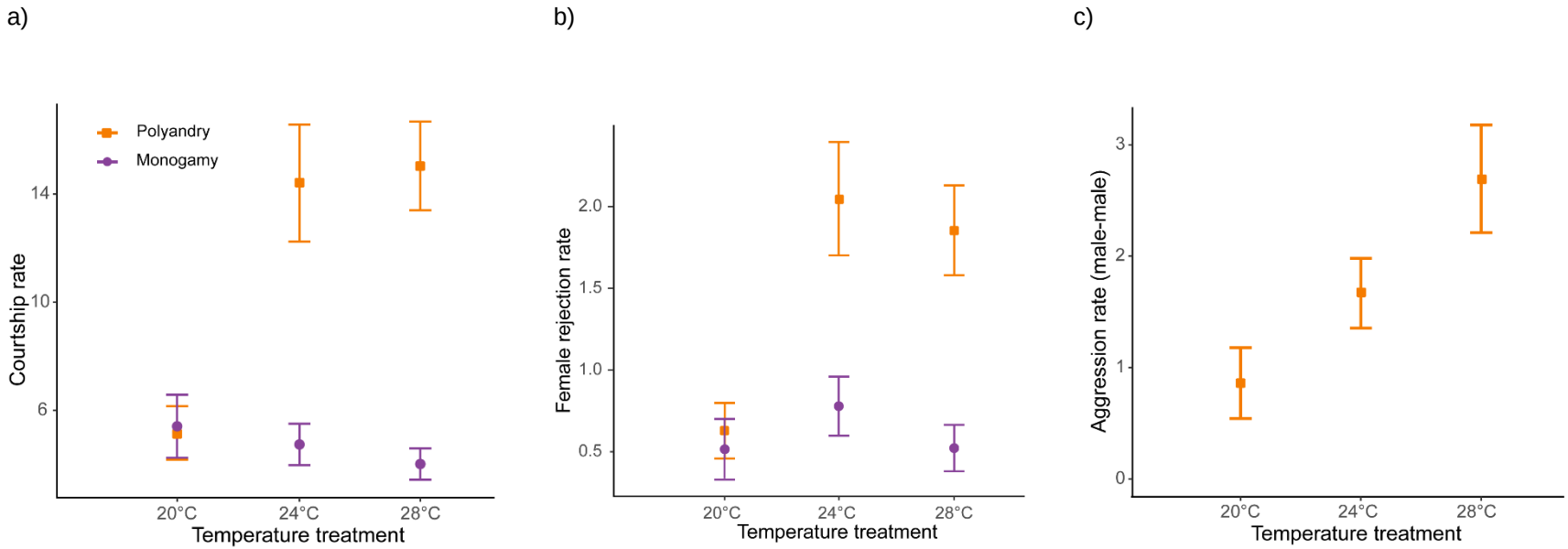
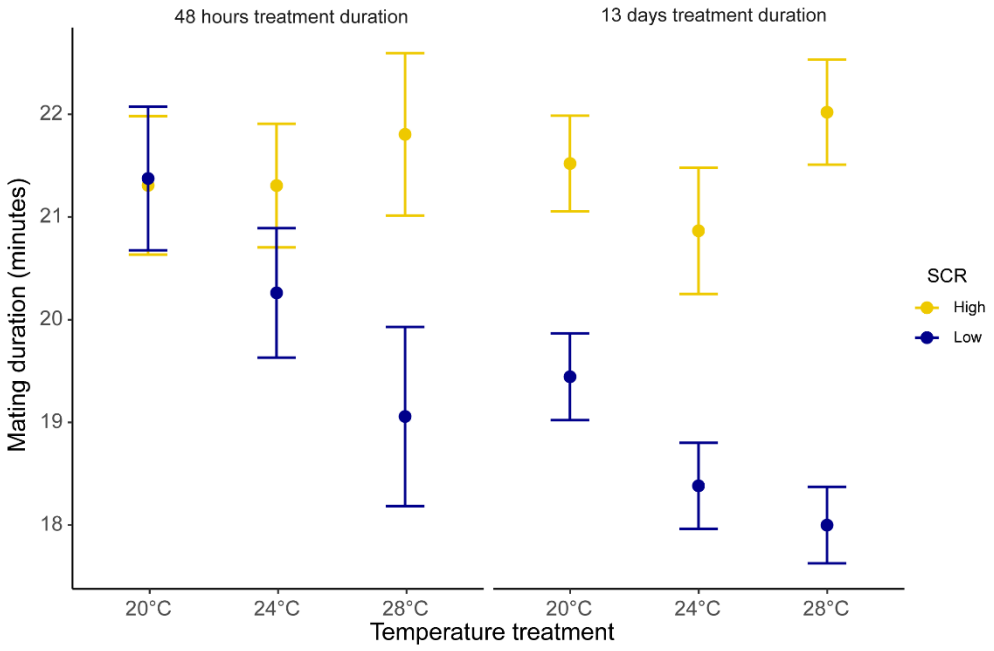



**Figure 5.** Reproductive behaviours (mean ± SEM) across temperature and mating system treatments. a) courtships per female per hour, b) female rejections per hour, and c) aggressions male-male per hour.

a)




b)

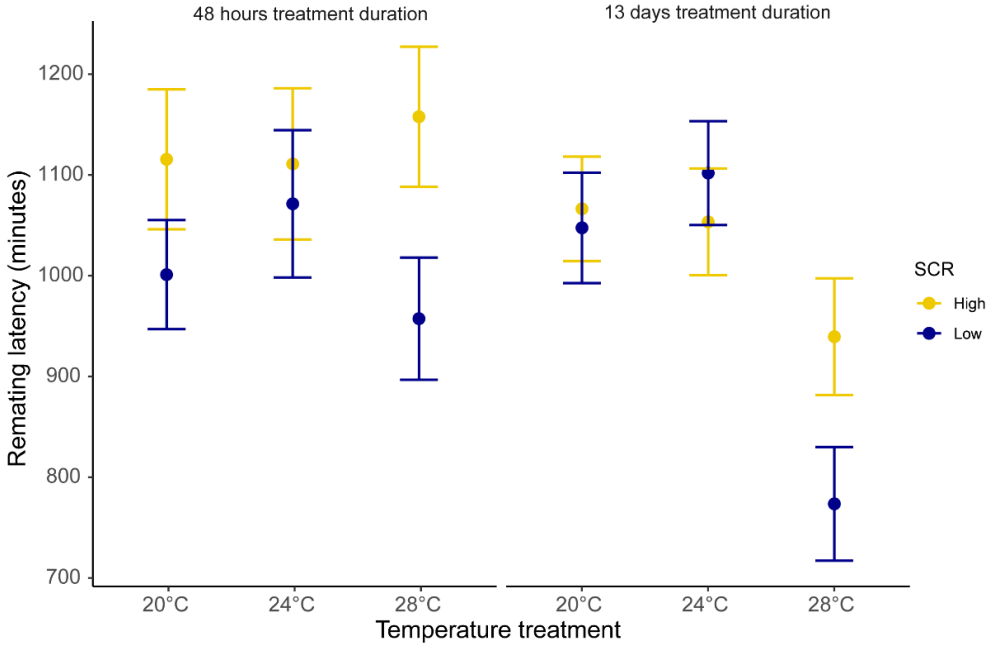



**Figure 6**. a) Mating duration (mean ± SEM) of males exposed to high (8 males per vial) or low sperm competition risk (1 male per vial) for 48 hours or 13 days prior to mating at different temperatures, b) female remating latency (mean ± SEM) following a single mating with either a male from a high or low sperm competition risk level for 48 hours or 13 days before mating across temperature treatments.