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Multiple mating rescues offspring sex ratio but not productivity in a haplodiploid exposed to developmental heat stress

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Abstract

1. Reproduction is generally more sensitive to high temperatures than survival and arguably a better predictor of the response of populations to climate change than survival estimates. Still, how temperature simultaneously impacts male and female reproductive success, the mating system and the operational sex ratio remains an open question.

2. Here, we addressed how a sublethal high temperature affects the reproductive system of the haplodiploid spider mite *Tetranychus urticae*. Males and females maintained at 25 or 36°C during development were paired and the fertility of both sexes, their mating and remating eagerness, and the paternity of the offspring of females with different mating histories were measured.

3. Female and male fertility decreased at 36°C compared to 25°C, resulting in lower offspring production and a more male-biased sex ratio, respectively, because of haplodiploidy. However, when either heat-stressed females or females that mated with heat-stressed males remated, there was a shift in paternity share, with more than one male contributing to the offspring. This was accompanied by reduced mating eagerness in pairs with partially sterile males and increased remating eagerness in pairs in which at least one sex was partially sterile in the first mating.

4. The observed temperature-induced changes in female remating eagerness and sperm use allowed restoring the offspring sex ratio, by increasing the proportion of fertilized offspring, but did not lead to the recovery of offspring number.

5. The temperature-induced changes in the mating behaviour and mating system should alter the interactions within and between the sexes, and with it the strength of sexual selection and sexual conflict in this species. Whether such changes are sufficient to prevent population extinction, despite the inability to recover offspring number, remains an open question.

KEYWORDS
climate change, fertility, mating behaviour, sperm use, spider mites

1 | INTRODUCTION

Climate change is leading to increased local temperatures and to more frequent heatwaves (Buckley & Huey, 2016; Coffel et al., 2018; Frölicher et al., 2018). These changes in the global thermal environment affect the biology of organisms at several levels, from behavioural and life-history traits of individuals to geographical range and abundance of populations, thereby posing a severe threat to biodiversity (Kellermann et al., 2012; Parmesan, 2006). This may be particularly detrimental to ectotherms, which represent the vast majority of terrestrial biodiversity (Stork et al., 2015), as their basic physiology is dependent on ambient temperatures (Hochachka & Somero, 2002).

Although viability has been often used as an indicator of species thermal limits (Geerts et al., 2015; Kellermann et al., 2012), it is becoming increasingly clear that thermal sensitivity differs among the main life-history traits, with male and female fertility being often the most sensitive traits in many taxa (Ma et al., 2015, 2020; Zhang et al., 2015; Zhao et al., 2014). Therefore, fertility is expected to be a better predictor of the response of populations to climate change than survival estimates, a prediction that is corroborated by recent studies (Parratt et al., 2021; van Heerwaarden & Sgrò, 2021; Walsh et al., 2019). Addressing the effect of these sublethal temperatures on reproductive traits is thus key to understand the impact of climate change on biodiversity.

Often, responses to sublethal high temperatures are sex specific (Iossa, 2019), with important and sometimes drastic evolutionary consequences (García-Roa et al., 2020; Leith et al., 2022). For instance, in the butterfly *Bicyclus anynana*, high developmental temperature affect the costs and benefits of mating in opposite ways in males and females, such that changes in sexual roles and ornamentation are observed (Prudic et al., 2011). Also, in several insect species, males are affected at lower temperatures than females, with male-induced sterility leading to changes in male–male competition (Vasudeva et al., 2014) and to females becoming more eager to mate to compensate for less sperm being transferred (Baur et al., 2022; Berger et al., 2011; Sutter et al., 2019; Vasudeva et al., 2021). Most studies exploring the effects of thermally induced sterility on mating behaviour and reproductive success concern polyandrous species (Baur et al., 2022; Berger et al., 2011; Prudic et al., 2011; Vasudeva et al., 2014, 2021), with a single study using a monandrous species (Sutter et al., 2019). This is unfortunate because the mating system has been shown to influence male and female thermal sensitivity in different species (e.g. Baur et al., 2022; García-Roa et al., 2019; Martinossi-Allibert et al., 2019; Parrett & Knell, 2018), possibly modulating population responses to environmental change (Candolin & Heuschele, 2008). Furthermore, in monandrous species, or in species with single paternity, multiple paternity caused by exposure to high temperatures implies a change in the mating system, which should result in a greater rearrangement of the selection pressures felt by males and females in these species than in polyandrous species (García-Roa et al., 2020). It is thus harder to predict how temperature will affect sexual selection and conflict, and how these, in turn, will impact the population viability of monandrous species.

Differential responses to sublethal high temperature between the sexes can also result in cryptic shifts in the operational sex ratio (OSR), as observed in some insect species (García-Roa et al., 2020), including in *Drosophila virilis* (Walsh, Parratt, et al., 2021). In this species, male, but not female, sexual maturation is delayed at high temperature, with the OSR of populations becoming more female-biased without observable changes on the adult sex ratio (ASR; Walsh, Mannion, et al., 2021). Alternatively, changes in OSR can be accompanied by changes in ASR in species with temperature-dependent sex determination and in haplodiploids (Maurer et al., 2021; Mobley & Jones, 2009). Specifically, in haplodiploids, female sterility results in reduced offspring production but male sterility leads to a male-biased ASR, because fertilized eggs only generate (diploid) daughters, while (haploid) sons are generated from unfertilized eggs (Helle & Sabelis, 1985). In these cases, heat-induced sterility could result in changes in both sex ratio and productivity. Yet, no study to date investigated the effect of sublethal temperatures on such species, despite the recognized impact of these variables on population dynamics and their role as drivers of plastic and/or evolutionary changes (Geffroy & Wedekind, 2020; Schmickl & Karsai, 2010).

Here, we study the effect of high sublethal temperatures on male and female fertility, paternity share and mating behaviour in the spider mite *Tetranychus urticae*, a ubiquitous agricultural pest (Migeon et al., 2010). Spider mites are exposed to temperatures above 35°C in different regions of the world (Caillon et al., 2014; Stavrinides et al., 2010). Particularly in Portugal, where the populations used were collected, especially during summer months, the time of the year in which high densities of spider mites are found in the field (Zélé, Santos, Godinho, et al., 2018), it can be extremely warm for extended periods (IPMA, 2022a). For instance, in August 2022, the average temperature felt across the country reached 29.9°C and the maximum temperature was 37.3°C, which lasted on average 11 days (IPMA, 2022a, 2022b). Therefore, addressing whether such high temperatures affect males and/or females in this species is an ecologically relevant research question.

Spider mites have a haplodiploid sex-determination system (Helle & Sabelis, 1985), typically with a female-biased ASR in the laboratory (ca. 70%–80% females; Mitchell, 1972). This species presents a pattern of nearly complete first-male sperm precedence, with mated females leaving the mating pool as soon as they effectively mate and for their whole lifetime (Helle, 1967; Rodrigues et al., 2020). This is not the case for males, which do not become sperm depleted unless they mate with more than ca. 15 females a day (Krainacker & Carey, 1989). This results in a male-biased OSR (Kokko et al., 2012). Under these circumstances, the costs of male sterility for females are thus likely to be high, as females mated to sterile males will still leave the mating pool permanently, but will not produce fertilized offspring, except if they change their pattern of sperm precedence. The mating behaviour of spider mites follows the expected behaviour of species with this sperm precedence pattern: males actively guard immature females to mate with them as soon

as they reach adulthood (Potter et al., 1976), they prefer virgin over mated females (Oku, 2014; Rodrigues et al., 2017) and have prolonged copulations to ensure sperm precedence (Satoh et al., 2001). Although the response of spider mites to temperature has been vastly documented (Farazmand, 2020; Gotoh et al., 2010; Praslička & Huszár, 2004; Riahi et al., 2013; Zou et al., 2018), to date no study has tested if the sperm precedence pattern in spider mites is disrupted, and with it, if reproductive behaviour is affected, in response to heat-induced sterility.

2 | MATERIALS AND METHODS

2.1 | Spider mite populations and rearing conditions

Two populations of spider mites (red form of *Tetranychus urticae*), one resistant and one susceptible to the pesticide etoxazole, were used in the experiments. These two populations share the same genetic background, except for the presence or absence of a single nucleotide recessive mutation on the chitin synthase 1 gene (CHS1) causing resistance to etoxazole (van Leeuwen et al., 2012). Briefly, the procedure used to create these two populations was as follows. Both populations originated from the same five population replicates (the 'iC' populations in Rodrigues et al., 2022), themselves formed by merging six *Wolbachia*-infected, homozygous etoxazole-susceptible, populations collected in the region of Lisbon, Portugal, in 2013 (Zélé, Santos, Olivieri, et al., 2018). Five etoxazole-resistant population replicates were then created by performing eight generations of backcross between each of the susceptible population replicates, and a homozygous etoxazole-resistant paternal line *SB9.Rif* (originally collected in Crete, Greece, in 2006; van Leeuwen et al., 2012). Each generation of backcross was followed by a generation of selection (with etoxazole exposure) of the haploid males. This procedure allowed the complete introgression of the etoxazole-resistant allele into the susceptible population replicates, but with complete retention of their mitochondrial genome and an estimated retention of 99.6% of their nuclear genome. The presence or absence of mutation of the CHS1 gene in all susceptible and resistant population replicates was confirmed by PCR-RFLP (as described in van Leeuwen et al., 2012). All population replicates were then treated with rifampicin following the procedure described in Zélé et al. (2020). Both susceptible and resistant replicates were merged in September 2020 to create, respectively, the susceptible (*Wu.SS*, uninfected with *Wolbachia*, susceptible to the pesticide) and resistant (*Wu.RR*, uninfected with *Wolbachia* and resistant to the pesticide) base populations used here.

The two base populations were maintained in large numbers (>2000) under continuous generations on entire plants from September 2020 to January 2021, then in smaller numbers (>1000) and with discrete generations from January 2021 onwards (to facilitate obtaining individuals of a specific stage for the experiments). These populations were kept on bean leaves (*Phaseolus vulgaris*, variety Contender, provided by Germisem, Portugal) under controlled conditions (25°C; 16 h light: 8 h dark photoperiod). Bean plants were germinated and grown in an isolated and herbivore-free room for 14 days under controlled conditions (16 h light (at 25°C): 8 h dark (at 20°C) photoperiod) before being exposed to spider mites.

2.2 | Experimental setup

2.2.1 | Fertility and paternity share

To test whether the offspring paternity share is altered when the first male has reduced fertility due to developmental heat stress, we crossed a resistant female (from *Wu.RR*) with a susceptible male (from *Wu.SS*) and then with one or several resistant males (from *Wu.RR*), following a protocol developed in Rodrigues et al. (2020). Paternity share was determined by exposing the resulting offspring to the pesticide etoxazole. This pesticide inhibits the synthesis of chitin, preventing eggs and juveniles from hatching and moulting, respectively, and resistance is determined by a single recessive allele (van Leeuwen et al., 2012). Because spider mites are haplodiploid and females always came from the resistant population, the pesticide application will only affect daughters (diploids), while sons (haploids), which only inherit the genetic material of their mothers, will always be resistant. Thus, when exposed to pesticide during development, the offspring will consist of alive sons, alive daughters (sired by resistant males) and/or unhatched eggs (sired by susceptible males).

Four treatments, differing in the temperatures under which females and their first mate developed, were tested: pairs where both individuals developed at control temperature (25°C; ♀25 × ♂25), pairs where both individuals were exposed to heat stress during development (36°C; ♀36 × ♂36), and pairs where only the male or the female was exposed to heat stress (♀25 × ♂36 and ♀36 × ♂25, respectively). These treatments were orthogonally crossed with mating history treatments, in which females from all developmental temperature treatments mated once, twice or multiple times. The second and subsequent matings always involved control males.

To obtain females from all developmental temperature treatments and mating histories, virgin males and females were taken from cohorts of each base population kept at either 25 or 36°C (control temperature and heat stress, respectively). Five resistant virgin females and five susceptible virgin males were placed on a mating disc (bean discs of 0.5 cm² placed inside a Petri dish with water-saturated cotton) at 25°C to mate for 30 min. After each mating, the males were removed to prevent further matings, and females were either placed on a leaf disc of 0.95 cm² without males (single mated females, SM; total of 5 females per disc) or transferred to a new mating disc (0.5 cm²) with five resistant virgin males developed at control temperature and given 2 h to remate (double mated females, DM; total of 5 females per disc). Again, once remated, females were placed in groups of 5 on leaf discs of 0.95 cm², while males were discarded after their first mating. Both matings were observed in order to verify that copulation occurred, but no data were recorded. A subset of the SM females was transferred in groups of 5 to a larger mating disc (0.95 cm²)

with 5 resistant virgin males that developed at control temperature, where they could freely remate multiple times and/or with multiple males for 24 h (multiply mated females, MM). Moving females to larger mating discs reduced the risk of accidental death by drowning, without compromising the mating frequency, as young virgin males can mate up to 15 times a day (Krainacker & Carey, 1989) and, for our experiment to work, only one mating needed to occur in these patches. The next day, single-, double- and multiply-mated females were individually transferred to new discs (2.55 cm^2) placed on etoxazole-soaked cotton (diluted in water at 0.5 g/L). Females had 4 days to oviposit, after which they were discarded, and the eggs were counted. The survival status of each female was recorded every day until female removal. The number of hatched and unhatched eggs was recorded 8 days after oviposition started. Adult offspring was sexed, counted and removed 12 and 14 days after the onset of oviposition.

This experiment was divided in 4 blocks, 2 per week in consecutive days, each including all treatments. SM females that did not mate and DM females that did not remate were discarded. Replicates with damaged females (i.e. females that were hurt during manipulation) were excluded from the analysis. Overall, we analysed 127 replicates from treatment ♀25×♂25, 126 replicates from treatment ♀25×♂36, 109 replicates from treatment ♀36×♂25 and 113 replicates from treatment ♀36×♂36 (see Table S1 for more details).

2.2.2 | Mating behaviour

To determine whether female mating and remating eagerness were affected by developmental heat stress, four mating treatments, targeting focal females and their first mate, were established: pairs where both individuals developed at control temperature (25°C; ♀25×♂25), pairs where both individuals were exposed to heat stress during development (36°C; ♀36×♂36) and pairs where only the male or the female was exposed to heat stress (♀25×♂36 and ♀36×♂25, respectively). Individuals were collected from cohorts of the base population *Wu.RR* kept at 25 or 36°C during development (control temperature and heat stress, respectively).

Virgin females and males were paired on a mating disc (0.5 cm^2) placed in a Petri dish with water-saturated cotton and given 30 min to copulate. The latency to copulation and its duration were recorded using the app Stopwatch: StopNow Free. To avoid unconscious user biases, a colour code unknown to the observer was established for each treatment prior to the mating observations. Once mated, females were either individually placed on leaf discs of 0.95 cm^2 (SM females) or transferred to a new mating disc (0.5 cm^2) to which five virgin males that had developed at 25°C were added. Mated spider mite females tend to take longer to remate at optimal temperature (Rodrigues et al., 2017), so pairs were given 2 h to mate instead of 30 min, enough time for remating to occur in this species (Rodrigues et al., 2020). Every time a copulation ended, males were removed from the patch and replaced by virgin ones, to ensure mating eagerness. Both latency to

each copulation and copulation duration were recorded. Afterwards, these females were individually placed on leaf discs of 0.95 cm^2 (MM females). The next day, both SM and MM females were individually transferred to larger leaf discs (2.55 cm^2) and their offspring was monitored as above.

This experiment was performed in 8 blocks, 2 per week in consecutive days, each including all treatments. Individuals were maintained at 25°C during the mating observations and afterwards. Replicates with single mated females that did not mate, multiply mated females that did not remate or damaged females (i.e. females that were hurt during manipulation) were excluded from the analysis. Overall, we analysed 361 replicates to determine the effect of developmental heat stress on female latency to the first mating, 260 replicates to test that effect on female duration of the first mating and 133 replicates to test that effect on female remating eagerness (both latency to and duration of second mating; see number of replicates per treatment in Table S1).

2.3 | Statistical analyses

All statistical analyses were performed using the software R (version 4.0.3; R Core Team, 2020). We performed generalized mixed-effects models (GLMM) and linear mixed-effects models (LMM) implemented in LME4 (version 1.1.26; Bates et al., 2015) and GLMMTMB (version 3.3.3; Brooks et al., 2017), and mixed effects cox models, implemented in COXME (version 2.2-16; Therneau, 2020), depending on the data and error structure. For discrete variables, overdispersion was tested by calculating the sum of squared Pearson residuals and comparing it to the residual degrees of freedom. Maximal models were simplified by removing non-significant terms ($p > 0.05$) from the highest-order to the lowest-order interaction (Crawley, 2012; Tables S1 and S2). The significance of each explanatory variable was determined by chi-squared tests for discrete distributions, and Wald F tests for continuous distributions (Bolker et al., 2009). *A posteriori* contrasts with Bonferroni corrections were made to interpret the effect of factors with more than two levels, using the EMMEANS package (version 1.5.3; Length, 2020; Tables S3 and S4). Graphical representations of the data were produced with the software package GGPLOT2 (version 3.3.3; Wickham, 2016).

2.3.1 | Fertility and paternity share

To test how developmental heat stress affected fertility and paternity share, three variables were analysed (Table S2): total offspring number (i.e., sum of the number of daughters, sons and unhatched eggs per female), as a measure of female fertility, the contribution of all males to the offspring (corresponding to the offspring sex ratio calculated as the proportion of daughters, i.e., the number of daughters and unhatched eggs, over the total offspring number), and the contribution of the second and subsequent males to the fertilized

offspring (i.e., the proportion of daughters over the number of daughters and unhatched eggs).

The total offspring number was analysed using a GLMM with a quasi-Poisson error distribution (package GLMMTMB), to account for overdispersion. The contribution of all males to the offspring was computed using *cbind* (with the sum of daughters and unhatched eggs and the number of sons as arguments), and was analysed using a GLMM with a parameter that accounts for zero inflation (ziformula ~1; package GLMMTMB) and a beta-binomial error distribution to account for overdispersion. The contribution of the second and subsequent males to the offspring was computed using the function *cbind* with the number of daughters and the number of unhatched eggs as arguments and analysed using a GLMM with a binomial error distribution (package LME4).

The developmental temperature of the female (25 or 36°C) and of the first male (25 or 36°C), the female mating history (SM, DM or MM), as well as their interaction, were added as fixed factors in the models for the total number of offspring and for the contribution of all males to the offspring. In the model for the contribution of the second and subsequent males to the offspring, the developmental temperature of the pair (female and first male; $♀25×♂25$, $♀25×♂36$, $♀36×♂25$ and $♀36×♂36$), the female mating history (SM, DM or MM) and their interaction were included as fixed factors. The developmental temperature of the pair was used instead of the developmental temperatures of the female and of the first male separately to avoid convergence issues. As expected, when females were single mated, the proportion of daughters (i.e., the contribution of the second and subsequent males) was zero, with only one replicate having a proportion of daughters above zero (in treatment $♀25×♂36$; proportion = 0.034). To deal with this lack of variance that impeded the statistical analysis, we manually added one replicate to the SM mating history of each treatment: the values of these replicates were equal to the one replicate above zero in treatment $♀25×♂36$, this way solving the variance problem while maintaining the original differences among treatments. Block, temperature and humidity during mating observations were included as random factors in all models (Table S2).

2.3.2 | Mating behaviour

To analyse how mating and female remating eagerness were affected by developmental heat stress, we analysed latency to the first copulation and the duration of that copulation, the latency to the second copulation and the duration of that copulation (Table S3). The number of adult offspring and sex ratio were also analysed to infer female and male fertility, respectively, and to confirm replicability with the results obtained in the previous experiment (cf. Supporting Information 1).

The latency to the first and to the second copulations were analysed using mixed effect cox models (package COXME). The duration of both first and second matings was analysed using LMMs with a Gaussian error distribution (package LME4). The data were boxcox transformed (duration of first copulation: $\lambda = 0.39$; duration of second copulation: $\lambda = -0.05$; MASS package; version 7.3.53; Venables & Ripley, 2002) to improve the model fit.

In all models, block and temperature and humidity during mating observations were included as random factors, and the developmental temperature of the female (25 or 36°C) and of the first male (25 or 36°C), as well as their interaction, were added as fixed factors. In addition, the mating rate (i.e., the number of copulations each female experiences) was included as a covariate in the models for the latency to the second copulation and its duration, as these traits might be affected by female eagerness to remate (Table S3).

3 | RESULTS

3.1 | Fertility and paternity share

The total number of offspring was affected by the interaction between the developmental temperature of females and of their first mate ($\chi^2_1 = 4.608$, $p = 0.032$; Table 1), but not by the female mating history ($\chi^2_2 = 1.782$, $p = 0.410$; Table 1). Indeed, thermally stressed females produced ca. 74% fewer offspring than control females (9 ± 0.52 offspring at 36°C and 35 ± 0.84 at 25°C), independently of the developmental temperature of their mates or their mating history (Table S4; Figure 1a). In addition, control females produced ca. 15% fewer offspring when first mating with thermally stressed males (32 ± 0.98) than when mating with control males (38 ± 1.32; Table S4; Figure 1a).

The contribution of all males to the offspring (i.e., the offspring sex ratio calculated as the proportion of daughters) was significantly affected by the interaction between the developmental temperature of the first male and the mating history of the female ($\chi^2_2 = 27.528$, $p \leq 0.001$; Table 1) and between the developmental temperature of the female and of her first mate ($\chi^2_1 = 4.510$, $p = 0.034$; Table 1). As expected, in control males, there was no significant effect of mating history, with the proportion of fertilized offspring averaging 0.70 ± 0.03 (Table S4; Figure 1b). Moreover, the proportion of fertilized offspring decreased when the female and/or the first male she mated with were exposed to heat stress. The proportion of fertilized offspring of females that mated once with heat-stressed males was reduced by 0.41 ± 0.08 compared to that of females mated once with control males (Table S4; Figure 1b). However, when these females were allowed to remate, the proportion of fertilized offspring increased, being the highest when females mated multiply (Table S4; Figure 1b), in which case it reached values close to those of females mated with control males.

The contribution of the second and subsequent males to the offspring was significantly affected by the interaction between the female mating history and the developmental temperature of the pair ($\chi^2_6 = 22.956$, $p \leq 0.001$; Table 1). As expected, the proportion of offspring sired by the second or subsequent males was the lowest when the females and their first mates developed at control temperature (Table S4; Figure 2). When at least one of the sexes involved in the first mating was exposed to developmental heat stress, the paternity share of subsequent males increased significantly, ranging from ca.

Dependent var.	Explanatory var.	df	χ^2	p-value
Total offspring number	♀ **temperature**	1	269.271	**<0.001**
	♂ **temperature**	1	7.376	**0.006**
	♀ mating history	2	1.782	0.410
	♀ **temperature × ♂ temperature**	1	4.608	**0.032**
	♀ temperature × ♀ mating history	2	0.032	0.984
	♂ temperature × ♀ mating history	2	2.877	0.237
	♀ temperature × ♂ temperature × ♀ mating history	2	2.477	0.290
Contribution of all males to the offspring	♀ **temperature**	1	12.420	**<0.001**
	♂ **temperature**	1	11.543	**<0.001**
	♀ **mating history**	2	7.248	**0.027**
	♀ **temperature × ♂ temperature**	1	4.510	**0.034**
	♀ temperature × ♀ mating history	2	3.138	0.208
	♂ **temperature × ♀ mating history**	2	27.528	**<0.001**
	♀ temperature × ♂ temperature × ♀ mating history	2	5.343	0.069
Contribution of the second and subsequent males to the offspring	**Temperature of pair**	3	516.785	**<0.001**
	♀ **mating history**	2	53.679	**<0.001**
	Temperature of pair × ♀ mating history	6	22.956	**<0.001**

TABLE 1 Statistical results of the effect of temperature on fertility and paternity share. 'df': degrees of freedom. 'χ^2': Chi-square value obtained in each analysis. 'Total offspring number': number of daughters, sons and unhatched eggs per female; 'Contribution of all males to the offspring': number of daughters and unhatched eggs, over the total offspring; 'Contribution of the second and subsequent males to the offspring': proportion of daughters over the fertilized offspring. '♀ temperature': developmental temperature of the females tested; '♂ temperature': developmental temperature of the first male that mated with a female; '♀ mating history': the number of times a female mated; 'Temperature of pair': developmental temperatures of the female and the first male crossed in each replicate. Statistically significant terms ($p \leq 0.05$) are represented in bold.
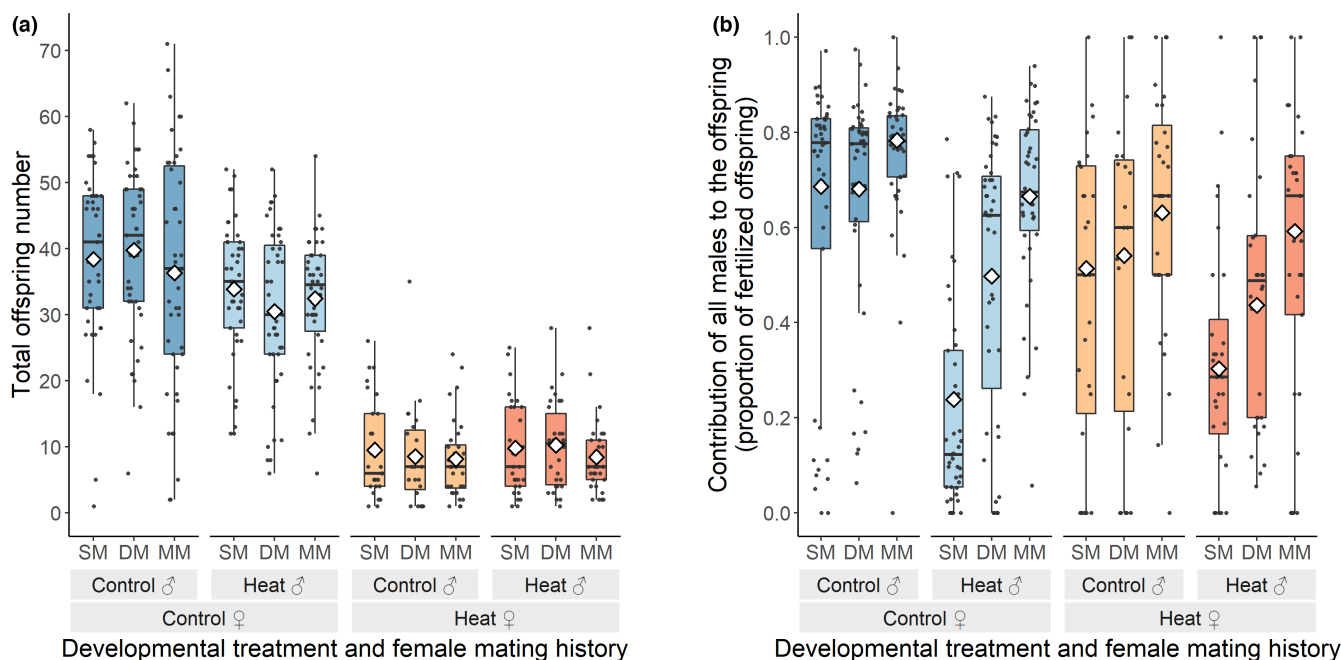

FIGURE 1 Effect of high developmental temperature on fertility and the contribution of all males to the offspring. Females mated once (SM), twice (DM) or multiply over 24 h (MM). The first mating involved a male susceptible to the pesticide etoxazole and developed at the temperature described in the figure (control = 25°C; heat = 36°C), while all subsequent matings involved control males resistant to etoxazole. (a) Total number of offspring, including sons, daughters and unhatched eggs, representing the fertility of the female; (b) Contribution of all males to the offspring, represented by the proportion of fertilized offspring. Boxplots display a median line, interquartile range (IQR) boxes, 1.5 × IQR whiskers and data points. Colours dark blue, light blue, light orange and dark orange represent treatments where both individuals were developed at the control temperature, only the male or the female were heat stressed, and both individuals were heat stressed, respectively. White diamonds represent the mean values per conditions tested.
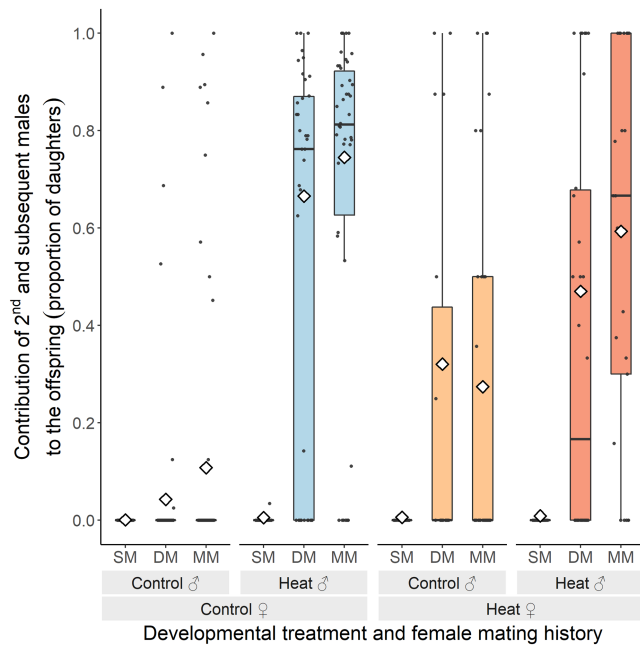
FIGURE 2 Effect of high developmental temperature on sperm precedence. Females developed at 25 (control) or 36°C (heat) mated once (SM), twice (DM) or multiply over 24 h (MM). The first mating involved a male susceptible to the pesticide etoxazole and that developed at the temperature described in the figure, while all subsequent matings involved males that developed at 25°C and were resistant to etoxazole. The contribution of second matings (and subsequent matings, in the case of MM) to the offspring is represented by the proportion of daughters (resistant to pesticide) over the total number of fertilized offspring. Boxplots display a median line, interquartile range (IQR) boxes, $1.5 \times$ IQR whiskers and data points. Colours dark blue, light blue, light orange and dark orange represent treatments where both individuals were developed at the control temperature, only the male or the female were heat stressed, and both individuals were heat stressed, respectively. White diamonds represent the mean values per conditions tested.

30%–70% of the fertilized offspring. Indeed, when the first male to mate with a female was exposed to heat stress, second and subsequent males contributed the most to the offspring, especially if the female developed at control temperature (Table S4; Figure 2). In addition, a significant increase of ca. 0.10 in the proportion of daughters when females mated multiply, compared to when they mated twice, was observed only in control females (Table S4; Figure 2).

3.2 | Mating behaviour

The interaction between the developmental temperature of both sexes affected the latency to the first copulation ($\chi^2_1 = 4.034$, $p = 0.045$; Table 2). Indeed, heat-stressed males, when compared to control males, took on average the double of the time to start copulating with control females and ca. 30% longer to copulate with heat-stressed females (Table S5; Figure 3a). The duration of the first copulation was only affected by the developmental temperature of

the male ($F_{1,246.94} = 13.866$, $p \leq 0.001$; Table 2), with pairs including heat-stressed males having ca. 20% shorter copulations than pairs including control males (Figure 3b).

The latency to the second copulation with a fertile male was affected by the developmental temperature of the first male to mate with the female ($\chi^2_1 = 13.719$, $p \leq 0.001$; Table 2), as pairs with females first mated to heat-stressed males took ca. 55% less time to start remating than pairs with females first mated to control males (Figure 3c). The duration of this second copulation was affected by the interaction between the developmental temperature of the female and of her first mate ($F_{1,119.398} = 8.548$, $p = 0.004$; Table 2): control females first paired with control males had the shortest copulation duration, with all other pairs involving at least one individual exposed to heat stress showing a larger duration (Table S5; Figure 3d).

4 | DISCUSSION

In this study, we showed that at high sublethal developmental temperature, not only was the fertility of both sexes hindered in spider mites, but the paternity share was altered, with rematings also contributing to the offspring. In accordance with the toll on fertility and change in paternity share, matings involving males that developed at high temperature took longer to occur and were shorter than matings with fertile males, while females involved in matings with partially sterile males were also more eager to remate with fertile males and rematings involving partially sterile individuals lasted longer. These changes resulted in a near total restoration of the proportion of fertilized offspring (i.e., daughters) but not in the recovery of the number of offspring produced (i.e., daughters and sons).

A reduction in fertility at high temperature has been previously demonstrated in many taxa for both sexes (Parratt et al., 2021; Paxton et al., 2016; Sales et al., 2021; Walsh et al., 2019), with accumulating evidences of deficiencies, unviability, depletion or incomplete transfer of sperm/ejaculate in males (Gillooly et al., 2001, 2002; Hurley et al., 2018; Lucio et al., 2016; Nguyen et al., 2013; Rahman et al., 2018; Rao et al., 2016; Sales et al., 2018; Setchell, 1998; Zeh et al., 2014) and changes in egg size and oocyte degeneration in females (Hansen, 2009; Paxton et al., 2016). Curiously, although spider mites are important crop pests known to be affected by temperature (Bayu et al., 2017; Kasap, 2004; Praslička & Huszár, 2004; Riahi et al., 2013), no study so far had disentangled the effects of heat stress on males and females in this group. By filling this gap, we found that male sterility resulted mostly in a shift in ASR towards more males and a slight decline (of ca. 15%) in offspring number. In line with the reduced reproductive success, matings with thermally stressed males showed increased latency to copulation and decreased overall mating duration compared to matings with control males. This suggests that exposure to high developmental temperature affects the mobility and/or constitution of males or their ability to perceive a female, which is supported by the fact that in the mating arenas some heat-stressed males seemed more prostrated than

TABLE 2 Statistical results of the effect of temperature on mating behaviour. 'df': degrees of freedom. 'F': sum of squares obtained from the F-test. 'Latency to the first copulation': time elapsed from pairing until the first mating of a female in seconds; 'Duration of the first copulation': time elapsed from the beginning until the end of the first mating of a female in seconds; 'Latency to the second copulation (remate)': time elapsed from pairing until second mating of a female in seconds; 'Duration of the second copulation (remating)': time elapsed from the beginning until the end of the second mating of a female in seconds. '♀ temperature': developmental temperature of the females tested; '♂ temperature': developmental temperature of the first male that mated with a female; 'Mating rate': number of matings done by multiple mated females; '♀ mating history': the number of times a female mated. Statistically significant terms (p ≤ 0.05) are represented in bold.

| Dependent var.	Explanatory var.	df (df residuals)	F	p-value
Latency to the first copulation	**♀ temperature**	**1**	**6.303**	**0.012**
	♂ temperature	**1**	**46.040**	**<0.001**
	♀ temperature × ♂ temperature	**1**	**4.034**	**0.045**
Duration of the first copulation	♀ temperature	1 (248.80)	1.100	0.295
	♂ temperature	**1 (246.94)**	**13.866**	**<0.001**
	♀ temperature × ♂ temperature	1 (245.48)	0.123	0.726
Latency to the second copulation (remate)	♀ temperature	1	2.557	0.110
	♂ temperature	**1**	**13.719**	**<0.001**
	Mating rate	**1**	**25.926**	**<0.001**
	♀ temperature × ♂ temperature	1	0.000	0.997
Duration of the second copulation (remating)	**♀ temperature**	**1 (117.555)**	**11.862**	**<0.001**
	♂ temperature	**1 (121.028)**	**30.959**	**<0.001**
	Mating rate	1 (110.23)	0.000	0.998
	♀ temperature × ♂ temperature	**1 (119.398)**	**8.548**	**0.004**
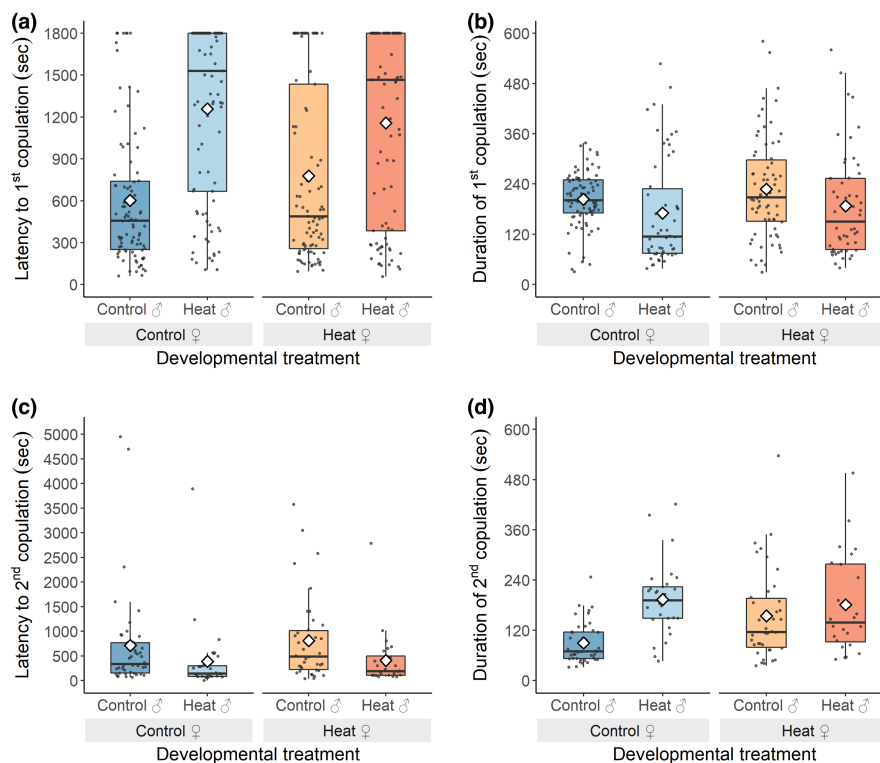
FIGURE 3 Effect of high developmental temperature on mating behaviour. Females mated once (SM) or multiply over 2h (MM). The first mating involved a male developed at the temperature described in the figure, while the subsequent matings involved control males. Latency to mate (a), duration of first copulation (b), latency to remate (female's second mating [c]) and duration of second copulation (d) are displayed in seconds. (a) and (b) include data from the first mating event of SM and MM females. (c) and (d) include data from the second mating event of MM females. Boxplots display a median line, interquartile range (IQR) boxes, 1.5 × IQR whiskers and data points. Colours dark blue, light blue, light orange and dark orange represent treatments where both individuals were developed at the control temperature, only the male or the female were heat stressed, and both individuals were heat stressed, respectively. White diamonds represent the mean values per conditions tested.

control males (personal observation). In addition, Tscholl et al. (2022) have shown that male spider mites exposed to fluctuating temperatures reaching 38°C suffer a decrease in body size. Both traits could affect mating behaviour (Enders, 1993; Li & Zhang, 2018). Another non-exclusive possibility is that females avoid mating with males exposed to high developmental temperature. A previous study in *T. urticae* has found similar behavioural changes in females involved in heterospecific matings that resulted in no fertilized offspring (Clemente et al., 2016), suggesting similar behaviours might be observed in different contexts due to different sources of infertility. In turn, here, heat stress in females translated simultaneously into reduced offspring number and an increase of ca. 15% more sons. Furthermore, no change in mating eagerness was observed between matings involving control vs heat stressed females, again in line with the results of Clemente et al. (2016) that found a lack of male preference for conspecific vs heterospecific females. Interestingly, when both sexes were exposed to heat stress, the ASR of the offspring was equivalent to that observed when only the male was heat stressed, and the number of adult offspring was equivalent to that found when only the female was exposed to heat, suggesting an additive effect of developmental heat stress of each parent on the offspring.

The effect of sublethal high temperature in males could have a snowball effect, as male sub-fertility results in the production of more (partially sterile) males. Moreover, the pattern of sperm precedence of this species is such that the first male generally sires all the offspring of a female (Helle, 1967; Rodrigues et al., 2020), suggesting that females engaging in matings with sub-fertile males might pay the high cost of not producing fertilized eggs. The combination of these two phenomena should rapidly lead to a highly male-biased sex ratio in the population. Adding to this the fact that females exposed to heat stress produced fewer offspring, the probability of population collapse due to this sublethal temperature seems to be high. This is particularly relevant because spider mites occur in fragmented populations in which females are the dispersing sex; hence, the number of females produced can be used as a measure of fitness (Metz & Gyllenberg, 2001). Thus, in a scenario with high temperatures, we expect fewer females to disperse to new plants, which can, in turn, result in the local extinction of populations.

The reduction in male and female fertility due to developmental heat stress has led, however, to the fertilization of some eggs by subsequent males, which resulted in an overall higher proportion of daughters being produced when females mated more than once. While we show that polyandry occurs, as the contribution of subsequent males to the female offspring ranged from ca. 30%–70%, we cannot exclude the possibility that the first mating was sometimes ineffective—that is, there was no sperm transfer—meaning that pseudopolyandry, and not polyandry, took place. Still, in most cases spider mites were clearly able to use the sperm of more than one male, which is a beneficial strategy at high temperatures since the recovery of the proportion of fertilized offspring owing to effective matings with more than one male was sufficient to reach values comparable to those found at optimal temperatures. Females that mated first with thermally stressed males also mated faster and

showed an increase in mating duration when remating with control males compared to females that had first mated with control males. This clearly shows that females modulate their mating behaviour. On the whole, the pre- and post-copulatory behaviour observed could help preventing population collapse, provided that females encounter fertile males via dispersal to, or migration from, cooler places. Two other studies in *Drosophila pseudoobscura* and *Tribolium castaneum* (Sutter et al., 2019; Vasudeva et al., 2021) have found similar changes in mating behaviour coupled with benefits of multiple mating after exposure to sublethal high temperature, whereas several others have found benefits of multiple mating against other sources of male sterility (Friesen et al., 2014; Gavriel et al., 2009; Kraaijeveld & Chapman, 2004; Landeta-Escamilla et al., 2016). This suggests that females can make dynamic remating decisions, with multiple mating functioning as a mechanism against different sources of failed matings or male infertility. This can help explain why multiple mating is so prevalent across species, including in those in which first male sperm precedence is the norm, like spider mites.

Unlike what was observed for the sex ratio and what has been documented in diploids (Sutter et al., 2019; Vasudeva et al., 2021), the fecundity of heat-stressed spider mites did not recover with multiple mating. This suggests that, in haplodiploids, multiple mating improves egg fertilization but not egg production. Furthermore, in heat-stressed females, the recovery of offspring sex ratio was lower, suggesting that these females are unable to fully store or use the sperm they receive. High temperature has been shown to affect egg size in females (Vasudeva et al., 2019) which in spider mites mediates sex allocation (Macke et al., 2012), giving a potential explanation for the inability to fully recover the offspring ASR. This might also explain why rematings with heat-stressed females lasted longer than those with control females. Whatever the mechanism at play, it seems that polyandry can be less beneficial to endure warming in haplodiploid species than in diploid species due to the decoupling of egg fertilization (dependent on male fertility) and egg production (dependent on female fertility).

Our findings have important consequences for the reproductive behaviour and the mating system of spider mites. Indeed, at optimal temperatures, the ASR of spider mite populations is typically female biased, with an average female proportion of circa 0.7 (Helle & Sabelis, 1985). In addition, males and females have different potential reproductive rates (Parker & Simmons, 1996), with most females leaving the mating pool permanently as soon as they mate, unlike males. This will result in a male-biased OSR (Kokko et al., 2012). Moreover, the Bateman gradient levels off after the first mating in females, whereas males either sire all or none of a female's offspring with every new mating, depending on whether they mate with a virgin or a mated female, resulting in a steep Bateman gradient with high variance. In contrast, under heat stress, here we show that females will remain in the mating pool after the first mating; hence, the OSR will approach the ASR. Also, because female multiple mating restores the sex ratio but not the offspring number, the Bateman gradient of females will remain unaltered as compared to optimal temperatures. In turn, males will sire fewer offspring per

mating, but this will occur regardless of the female mating status, which will reduce the variance in reproductive success between matings. This suggests that temperature has a sex-specific effect in the Bateman gradient of haplodiploids, unlike what would be predicted for diploids. Overall, these temperature-driven changes in ASR, OSR and Bateman gradient should strongly modulate the expression of sexual selection and conflict, as has been shown in other species in different contexts (Fitze & le Galliard, 2008; García-Roa et al., 2019, 2020; Janicke & Morrow, 2018; Li Richter & Hollis, 2021; Martinossi-Allibert et al., 2019; Perry & Rowe, 2018; Shuster, 2009). However, it remains unclear exactly how will this temperature-driven rearrangement in the dynamics of sexual selection and conflict affect population persistence. Ultimately, a novel research question arises: are haplodiploid species particularly vulnerable to climate change?

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.20069441.v1 (Costa et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Number of replicates analysed to test each variable of both the effect of temperature on fertility and paternity share and mating behaviour.

Table S2: Description of the statistical models used for data analysis of the effect of temperature on fertility and paternity share.

Table S3: Description of the statistical models used for data analysis of the effect of temperature on mating behaviour.

Table S4: *A posteriori* contrasts of significant explanatory variables of the effect of temperature on fertility and paternity share.

Table S5: *A posteriori* contrasts of significant explanatory variables of the effect of temperature on mating behaviour.

Supplementary Information 1: The effect of temperature on the offspring number and sex ratio of females from the mating behaviour experiment.