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Male and female developmental temperature modulate post-copulatory interactions in a beetle

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Running header: Developmental temperature and post-copulatory interactions
Abstract

Sexual selection theory has proven to be fundamental to our understanding of the male-female (sperm-egg) interactions that characterise fertilisation. However, sexual selection does not operate in a void and abiotic environmental factors have been shown to modulate the outcome of pre-copulatory sexual interactions. Environmental modulation of post-copulatory interactions are particularly likely because the form and function of primary reproductive traits appears to be acutely sensitive to temperature stress. Here we report the effects of developmental temperature on female reproductive architecture and the interaction between male and female developmental temperature on the outcome of sperm competition in the bruchid beetle *Callosobruchus maculatus*. When females were reared at developmental temperatures above and below typical temperatures the bursa copulatrix (site of spermatophore deposition) were smaller and, were either shorter and broader (high temperatures) or longer and thinner (low temperatures) than those reared at intermediate temperatures. Males and females reared at low developmental temperatures were less likely to mate than those reared at higher temperatures. Where copulation occurred, females reared at the highest temperature copulated for longest, whilst males reared at the lowest temperature spent longer in copula. Male developmental temperature had a significant impact on the outcome of sperm competition: males reared at 17°C were largely unsuccessful in sperm competition against control (27°C) males, although some of the variation in the outcome of sperm temperature. Our results demonstrate that male-female interactions that characterise pre- and post-copulatory outcomes are sensitive to developmental temperature and that plasticity in cryptic female preferences could lead to heterogeneous selection on the male reproductive phenotype.
1. Introduction

The reproductive phenotypes of males and females appear to be particularly sensitive to variation in temperature (Walsh et al., 2018) which, in turn, can affect the nature of the male-female interactions that characterise fertilization (García-Roa et al., 2020). The effects of temperature on reproductive phenotypes is especially evident in those species in which temperature determines the sexual phenotype (Charnier, 1966; Valenzuela & Lance, 2004). However, in most species the effects of developmental temperature on the form and function of primary reproductive traits are less pronounced than those characterised by temperature dependent sex determination and, as a consequence, are less well known. Studies on Drosophila by David et al. (1971) and Cohet (1973) were amongst the first to demonstrate that, above and below certain thermal thresholds, the fertility of males rapidly declines, primarily as a consequence of abnormal cyst elongation within the testes (Chakir et al., 2002; David et al., 2005). Since these early investigations, developmental temperature has been shown to affect sperm form and function across a variety of taxa (Walsh et al., 2018), including dung flies (Scatophaga stercoraria) (Blanckenhorn & Hellriegel, 2002), terrestrial snails (Arianta arbustorum) (Minoretti et al., 2013), guppies (Poecilia reticulata) (Breckles & Neff, 2013), and the beetles Callosobruchus maculatus (Vasudeva et al., 2014) and Tribolium castaneum (Sales et al., 2018). Male gametogenesis in plants is also particularly vulnerable to temperature stress (Müller & Rieu, 2016) to the point that just a few days of elevated temperatures can compromise crop yield (Sage et al., 2015).

The effects of developmental temperature on reproductive trait form and function are not restricted to males. In female plants, temperature stress can affect the development of the stigma and the style and the interactions between pollen grains and these transmitting tissues (Sage et al., 2015). In Scatophaga dung flies, the usual female reproductive phenotype is the three-spermatheca condition. However, when reared at elevated developmental temperatures the rarer four-spermatheca phenotype is expressed more frequently (Berger et al., 2011). This is particularly important within the context of post-copulatory sexual selection as spermathecal number in this species interacts with female quality to affect the outcome of sperm competition (Ward, 2000).

Despite the obvious importance of females in modulating the fertilization success of males (Eberhard, 1996), studies into the causes and consequences of temperature-related plasticity in female reproductive form and function are relatively rare. Moreover, the combined effects of variation in male and female developmental temperature on the outcome of post-copulatory sexual selection have yet to be reported. We argue that this is an important oversight because successful fertilisation is dependent upon sperm-female and/or sperm-egg interactions (Eberhard, 1996; Wilson et al., 1997; Miller & Pitnick 2002; Karr et al. 2009; Lupold et al., 2013; Higginson et al., 2012). Thus, studies into...
The plastic responses of a single sex may result in an incomplete understanding of the processes that characterise successful fertilization. This has important implications for concepts such as ‘thermal fertility limits’ (Walsh et al., 2018; Iossa, 2019) because a plastic response in the reproductive biology of one sex may change the fertility parameters of the other sex (see also Grazer & Martin, 2012; Bita & Gerats, 2013).

The effects of developmental temperature on primary reproductive trait form and function are likely to be widespread because the vast majority of animals are ectothermic, whereby developmental temperature is largely environmental. Some of the temporal variation in temperature will be relatively predictable, and may influence the production of seasonal polyphenisms (Whitman & Ananthakrishnan, 2009), whilst other temperature fluctuations are less predictable, but no less extreme. For example, insect larvae that complete their development in dung may experience temperature fluctuations in excess of 25°C over just a few days (Penttilä et al., 2013).

In the bruchid beetle *C. maculatus*, a model species for studies of post-copulatory sexual selection, the temperature at which males develop affects sperm size, sperm number, copulatory behaviour, and the outcome of sperm competition (van Leishout et al., 2013; Vasudeva et al., 2014, 2018). However, the effects of developmental temperature on the reproductive phenotype of female *C. maculatus* are unknown. This is important because copulatory behaviour, and competitive fertilization success in this species, are largely a product of male-female interactions (Wilson et al., 1997; Eady & Brown, 2017). Here, we report the effects of developmental temperature on female reproductive anatomy and behaviour and, for the first time, the combined effects of male and female developmental temperature on copulatory behaviour and the outcome of sperm competition.

2. Methods

(a) Study populations.

The population originated from Niamey, Niger and had been kept on black-eyed beans (*Vigna unguiculata*) for more than 200 generations at the University of Lincoln in an insectary held at approximately 27°C and 35% rh. Male and female *Callosobruchus maculatus* beetles were reared from the egg stage through to adult eclosion in incubators (Panasonic Cooled Incubator MIR-154 Series) maintained at 17°C, 27°C and 33°C. Vasudeva et al. (2014; 2018) found these temperatures to influence the reproductive behaviour and physiology of male *C. maculatus* and thus using the same temperatures here represents an important point of comparison in the consideration of the effects of temperature on the reproductive biology of females. This is particularly important because male reproduction appears to be more sensitive to thermal stress than female reproduction (Walsh et al. 2019), although as Iossa (2019) points out, a better understanding of the causes and consequences of
sex-specific sensitivity of fertility to temperature is pivotal when modelling the population consequences of heat stress. The upper temperature is well within the range experienced by populations of *C. maculatus* (Appleby & Credland, 2007), although it clearly negatively impacts male reproduction (Vasudeva et al 2014), whilst the lower temperature represents a value close to this populations lower thermal limit (Dalglish et al., 2021), although development can occur as low as 15°C (Mobarakian et al., 2014). To initiate these populations, approximately 200 newly eclosed adult beetles from either wild-type or black-morph stock populations, were placed into two separate containers holding approximately 200g each of moth beans (*Vigna aconitifolia*) to lay eggs for 24 hours at 27°C. Moth beans were used because, on average, only one adult beetle emerges per bean, thus allowing greater control over resource competition and the collection of virgin adults of known age is made easier. Egg-laden beans from these two populations were transferred to 150mm diameter Petri dishes and divided equally between the three temperature treatments. Approximately three days prior to adult eclosion, individual moth beans were transferred into the separate cells of 5 x 5 repli-dishes, fitted with glass lids, in order to isolate virgin adults following emergence. Dishes were checked daily for emergent adult beetles.

(b) Female genitalic morphology.

To assess the effect of developmental temperature on female reproductive morphology, virgin females from the three treatments were collected within 24h of eclosion and euthanised in a -20°C freezer. Female reproductive tracts were dissected free using watch-makers forceps and positioned in the left lateral plane on a microscope slide. An Olympus SZX12 dissection microscope was used to record a digital photograph using a Motic™ 1000 digital camera. In this plane, the bursa copulatrix and the bursal valve are clearly visible. Digital images were used to measure the length of the bursa from the distal end of the bursal valve to the end of the bursa copulatrix, and the breadth of the bursa copulatrix just under the bursal valve (Figure S1), using ImageJ (Schnieder et al., 2012). The length of the bursal valve was also measured along its longest axis and the width measured similarly across its widest axis. Female elytra length was measured as a proxy for body size (Wilson & Hill, 1989) using the methods described above.

Shape analysis of the bursa and bursal valves was applied to the same set of images in which tpsDig 2.17 (Rohlf, 2015) was used to place 150 and 50 evenly-spaced homologous semi-landmarks around the bursa and bursal valve, respectively. These digital coordinates were imported into MorphoJ v.1.60d (Klingenberg, 2011). Following a Procrustes transformation and generation of a covariance matrix, principal components analysis was performed to quantify variation in shape.
(c) Copulation.

Virgin males and females were collected 0-24h post-eclosion from the three temperature regimes and acclimatised for 24h at 27°C. In a room maintained at 27°C a single virgin female (reared at 17°C, 27°C or 33°C) was introduced to a single virgin male (reared at 17°C, 27°C or 33°C) under a Petri dish. Typically, 30 pairings from each temperature group were carried out but only a small number of females from the 17°C culture successfully copulated despite a large number of attempted matings (see results). In C. maculatus, females shorten the duration of copulation by kicking males about 2/3rd of the way through copulation (Edvardsson & Tregenza, 2005). The onset of kicking appears to be associated with ejaculate transfer: female kicking behaviour is initiated earlier when copulation is with a large male, which is associated with the transfer of a larger ejaculate (van Lieshout et al., 2014) whilst the kicking behaviour of females takes longer to elicit when females copulate with ejaculate-limited males (Eady & Brown, 2017). Copulation of each pair of beetles was observed and the duration of both the start-to-kick (Phase 1) and kick-to-end (Phase 2) phases (Eady, 1994) were recorded using a stopwatch.

(d) Sperm competition.

To determine the effects of developmental temperature on last-male sperm competitiveness, the genetic marker technique was used (Eady, 1991). Black-morph females derived from the 17°C, 27°C and 33°C, were mated in the insectary (27°C) to individual wild-type males (from the 27°C treatment) and 24-48 hours later, re-mated to a black-morph male, which had been reared at either 17, 27 or 33°C. Following the first copulation, females were placed in individual petri dishes containing approximately 30 moth beans to lay eggs. Following the second copulation the females were transferred to a second petri dish containing approximately 100 moth beans, on which she oviposited until her natural death. Both sets of eggs were maintained at 27°C until all viable offspring had emerged and paternity assigned based on offspring body colour (Eady, 1991).

(e) Statistical analysis.

Linear measures of bursal size and shape were analysed using analysis of covariance, with elytra length as the covariate. With regard to the geometric morphometric analysis of shape variation, lollipop plots were produced using MorphoJ to illustrate the movement of semi-landmarks in each of the principal components. PAST v2.17c (Hammer et al., 2001) was used to perform a one-way Non-Parametric Multivariate Analysis of Variance (NPMANOVA) analysis on Principal Component 1 and 2 (PC) scores to investigate shape variation in the bursae and bursal valves among the temperature treatments.
Copulation duration data were $\log_{10}$-transformed so that they approximated a normal distribution and were subsequently analysed via analysis of variance. The likelihood of mating was analysed using binary logistic regression with the binary division of copulate or not as the dependent variable and male and female developmental temperature entered as the independent variable in two separate models. $P_2$ data (the proportion of offspring sired by the second male to mate (Boorman & Parker 1976)) were analysed using binomial logistic regression, in which offspring from the 1st male to mate and offspring from the 2nd male to mate were bound together into a single response variable using cbind. The average number of offspring following a double mating was 36 (range 9 – 91). Male and female temperature and their interaction were entered as independent variables in a single model. To correct for overdispersion, F-tests were used (instead of chi-square) in the analysis of deviance (Crawley 2002). Because no 17°C females mated with 17°C or 33°C males (see results), the analysis was restricted to females reared at 27 and 33°C only. ANOVA and generalised linear models were performed in R version 2.15.2 (R Developmental Core Team., 2012).

3. Results

(a) Female genital morphology.

ANCOVA revealed no effect of the covariate female elytra length on bursal length nor width ($F_{1,55} = 0.12, p = 0.73$ and $F_{1,55} = 0.31, p = 0.58$, respectively) but did reveal a significant effect of developmental temperature on both measures ($F_{2,55} = 8.97, p < 0.0001$ and $F_{2,55} = 9.92, p < 0.0001$, respectively; Figure S2), with 17°C females expressing longer, thinner bursae, whilst those reared at 33°C had shorter, wider bursae. An examination of length x width² (an approximation of volume) revealed those females reared at the highest and lowest temperatures to have the smallest bursae: 17°C = 0.12mm³, 27°C = 0.19mm³, 33°C = 0.11mm³, $F_{2,55} = 10.39, p < 0.0001$, with a Tukey post-hoc test revealing the 27°C treatment to be greater than the 17 or 33°C treatments). Approximately 75% of the variation in bursal shape was explained by PC1 and PC2 combined, whereby PC1 can be characterised a widening/narrowing of the bursa copulatrix whereas PC2 describes a lengthening/shortening of the bursa copulatrix. NPMANOVA confirmed a significant effect of developmental temperature on bursal shape ($F_{2,57} = 9.53, p < 0.0001$; Fig 1); females that experienced low developmental temperature had longer, thinner bursae whilst those developing at 33°C had shorter, broader bursae (see also supplementary material); a result congruent with the linear measures of bursal shape presented above. Qualitatively similar effects were seen in the shape of the bursal valves, being longer and thinner at the lower developmental temperature (see supplementary material).
(b) Copulation.

Both males and females reared at 17°C were reluctant to mate (Fig 2): Binary logistic regression revealed a significant effect of both male ($X^2 = 121.1, P < 0.0001$) and female ($X^2 = 131.1, P < 0.0001$) developmental temperature on the likelihood of engaging in copulation. With 17°C females only engaging in copulation with 27°C males (Fig 2) it was not possible to analyse copulation duration data with a fully-factorial ANOVA. One-way ANOVA revealed a significant effect of female developmental temperature on the first phase of copula and the total duration of copula, with females reared at the highest temperatures engaging for longest in copula (Table 1; Figure 3). The developmental temperature experienced by males also affected the duration of copula: those reared at the lowest temperature spent significantly longer in the first phase of copula and in total (Table 1; Figure 3). These results were qualitatively similar when the copulation duration data were analysed using a fractional factorial design (the 27 and 33°C females copulated with the 17, 27 and 33°C males); essentially these analyses revealed 33°C females to spend longer in the first phase of copula and in total than the 27°C females, whilst 17°C males spent longer in all measures of copulation duration. No significant interaction terms were evident (see supplementary material).

(c) Sperm competition.

Males reared at 17°C performed particularly poorly in terms of sperm competition success (Fig. 4). However, success in sperm competition depended in part on the interaction between male and female developmental temperature (binomial logistic regression Δ deviance = 88.06, $F_{2,133} = 44.03$, $p < 0.0001$). Males reared at 17°C tended to perform best (in terms of $P_2$) when mating with 27°C females whilst males reared at 27°C and 33°C tended to achieve higher $P_2$ values when mated to 33°C females (Fig. 4). Removal of the interaction term revealed a significant effect of male and female developmental temperature on $P_2$: binomial logistic regression Δ deviance = 2699, $F_{2,137} = 1350$, $p < 0.0001$ and Δ deviance = 6.25, $F_{1,136} = 6.25$, $p < 0.012$, respectively. The interaction term was significant when the analysis was performed on only those matings that yield some last-male offspring (i.e., excludes the possibility that low $P_2$ is due to failure to transfer an ejaculate): female temperature*male temperature, Δ deviance = 26.46, $F_{2,110} = 26.46$, $p < 0.0001$.

4. Discussion

Where fertilization is internal, the anatomy and physiology of the female reproductive tract sets the rules by which sperm competition is played out (Eberhard, 1996; Wilson et al., 1997; Miller & Pitnick 2002; Karr et al. 2009; Lupold et al., 2013; Higginson et al., 2012). The extent to which these female-derived rules impact the evolution of primary reproductive traits is evident from the numerous
comparative studies performed on arthropod taxa that report tight associations between female reproductive architecture and sperm size (Dybas & Dybas, 1981; Pitnick et al., 1999; Presgraves et al., 1999; Morrow & Gage, 2000; Minder et al., 2005; Rugman-Jones & Eady 2008). Indeed, the length of the seminal receptacle in females appears to be the main driver behind the evolution of the extraordinarily long sperm of Drosophila bifurca (Lüpold et al., 2016) However, despite the importance of female reproductive architecture, relatively few studies have examined the evolved, or plastic, responses of female reproductive architecture per se in response to environmental heterogeneity.

The bursa copulatrix of females reared at the highest and lowest temperatures were smaller than those reared at the more typical temperature. Additionally, bursal shape was longer and thinner when female development took place at 17°C. The male ejaculate is delivered to the bursa copulatrix (Eady, 1994; Dougherty & Simmons 2017) and so changes in its size and/or architecture are likely to hamper the ability of males to correctly deposit and/or form their spermatophores within this structure. This may explain why females that experienced the highest developmental temperature spent longer in copula because previous research on C. maculatus has shown sperm-limited males and thermally-stressed males take longer to transfer smaller ejaculates. Thus, difficulties in the transfer and formation of ejaculates in this species translates to longer copulations (Vasudeva et al., 2018; Eady & Brown, 2017). This is thought to be a consequence of both males and females responding to a succession of exogenous and endogenous stimuli experienced during copulation (Eady & Brown, 2017). Thus, changes to the female reproductive phenotype (i.e., shape/size of the bursa copulatrix) in response to variation in developmental temperature are likely to dislocate these fine-tuned, male-female interactions, ultimately resulting in an extended duration of copula.

Previous studies into the functional significance of variation in copulation duration have reported elevated levels of damage (puncturing) of the female reproductive tract in relation to longer durations of copula (Crudgington & Siva-Jothy 2000; Edvardsson & Tregenza 2005). Subsequent studies revealed the extent of damage incurred by females to be related to male genital spine length which was associated with success in sperm competition (Hotzy & Arnqvist 2009; Hotzy et al. 2012), although male genital spine length was not associated with copulation duration (Hotzy et al. 2012). This may explain why Eady (1994) found no strong evidence for an association between copulation duration and sperm competition success in this species (see also Edvardsson & Tregenza, 2005).

Future studies utilising intraspecific variation in bursal size and/or shape might be able to shed more light on the mechanisms that drive variation in copulatory behaviour in C. maculatus.

Why the shape and size of the bursa copulatrix varies in relation to developmental temperature is open to conjecture. Temperature constrains the rates of biochemical reactions resulting in thermal sensitivities of function at cellular, systemic and organismal levels (Angilletta
Temperature can affect the rate of conformational change in proteins and the fluidity of cell membranes which in turn can act across ontogenic stages to shape adult phenotypes (Abram et al., 2017). Thus, distinguishing between adaptive plasticity (i.e., functional benefits to changes in phenotype in direct response to temperature perception), or changes in the phenotype driven by the kinetic effects of temperature on the insect’s physiological state, is difficult and beyond the scope of this study.

Similar to results reported by Vasudeva et al. (2014) males reared at 17°C did particularly poorly during sperm competition in comparison to those reared at 27°C or 33°C. However, success in sperm competition was in small-part due to the interaction between male and female developmental temperature. The mechanisms that underlie this interaction are currently unknown, although morphological and physiological plasticity in the female reproductive phenotype could influence fertilization success via male-female, ejaculate-female, or sperm-female interactions (Pitnick et al., 2009). For example, males copulating with females with abberant bursae may find it difficult to transfer an ejaculate or if successful, position it correctly within the female bursa. Within the bursa copulatrix, tiny bursal teeth are thought to pierce the membranous spermatophore (Dougherty & Simmons 2017), releasing sperm into the female reproductive tract that ultimately move through the female reproductive tract to be stored in the spermatheca. Misalignment of the spermatophore within the bursa could prevent or restrict the timely release of sperm from the spermatophore, compromising their long-term storage in the spermatheca.

The ramifications of these developmentally derived interactions are important at a number of levels. Firstly, ectothermic animals frequently encounter large fluctuations in developmental temperature (Penttilä et al., 2013; Burger, 1976). Thus, our finding that male and female developmental temperatures affect male-female reproductive interactions are likely to be applicable across a wide variety of taxa. Secondly, the demonstration that the temperature experienced by females during development can influence both the arena in which sperm competition is played out and its outcome, points to a mechanism by which variation in female-generated biases in sperm use can maintain genetic variation in male traits highly relevant to fitness, such as sperm length (Lüpold et al. 2016; Kelly, 2018). If genotype x environment interactions determine the nature of the female-derived environment in which sperm competition is played out, then what constitutes an optimal sperm phenotype could vary with the vagaries of the developmental environment (see also (Morrow et al., 2008). It should also be stressed that we have studied the effect of developmental temperature on one aspect of the female reproductive tract. These are complex physiological arenas that present a host of opportunities (e.g., the female reproductive proteome McCullough et al 2020) for external stressors to impact their function. However, our results are indicative only, in so much that we have
demonstrated the principle that male and female developmental temperatures interact to modulate the outcome of competitive fertilization under laboratory conditions. The lower temperature used here (17°C) is permissive for development but may be below the threshold for population growth (Dalglish et al., 2021). In effect, the evolutionary relevance of the interactions we have shown (beyond proof of concept) may be limited, although this will be resolved by further study into the effects of thermal stress above the threshold for population growth on developmental plasticity.

Our finding that the developmental temperature experienced by males and females affects the outcome of post-copulatory sexual selection resembles those pre-copulatory mate choice studies that have shown developmental temperature to influence female responses to male courtship signals (Rodríguez & Greenfield, 2003; Narrawayc et al., 2010). It is also important to note that the temperature experienced at the time of the interactions (i.e., ambient temperature) can also modulate the operation of sexual selection (García-Roa et al., 2018). For example, in the cigarette beetle (Lasioderma serricorne) ambient temperature affected the duration of copulation, the number of sperm transferred and the outcome of sperm competition (Suzaki et al., 2018). Thus, the kinetic effects of temperature can operate in the short-term and long-term (i.e., developmentally) (Abrams et al., 2017) to affect the outcome of post-copulatory sexual selection.

Understanding how temperature affects pre- and post-copulatory interactions is important in an era of global warming (Walsh et al., 2018; Medhaug et al., 2017). Fertility, the ability of an organism to produce viable offspring (Walsh et al., 2018), responds like many other behavioural, physiological and life-history traits to variation in temperature (Angilletta, 2009), whereby performance drops off sharply as thermal maxima and minima are reached. Thus, prior to reaching critical thermal limits, organisms rapidly lose fertility, rendering populations susceptible to extinction (Walsh et al., 2018). We argue that these population specific thermal fertility limits are likely to be intensified by disruptions to the numerous male-female interactions that typify successful reproduction. Thus, the use of proxies of fertility that are associated with one sex only, such as sperm motility, may underestimate the combined effects of thermal stress on population fertility (Iossa, 2019).

Here we show developmental temperature to affect both the female reproductive phenotype and the outcome of sperm competition. Plasticity in female reproductive traits in response to larval nutrition has been reported in a number of studies (Amitin et al., 2006; Martel et al., 2011; Cayetano & Bonduriansky, 2015) and shown to influence the outcome of sperm competition in Drosophila melanogaster (Amitin et al., 2006). Phenotypic plasticity has been argued to promote the origin of novel phenotypes, divergence among populations and subsequently reproductive isolation (Pfennig et al., 2010; Moczek et al., 2011). Plasticity in primary reproductive traits could represent an important
category of traits within this conceptual framework as environmentally derived variation in one sex will have consequences for the reproductive function of the other sex (Eberhard, 1996; Miller & Pitnick, 2002; Kelly, 2018; Arnvist & Rowe, 2005). Should this plasticity be associated with a particular set of environmental conditions then consistent selection could result in the evolution of habitat specific primary reproductive traits, a potential pre-cursor to incipient reproductive incompatibility. Further studies are required to determine how variation in temperature affects the plastic (short-term) and evolved (long-term) responses of reproductive traits and, in an era of global warming, how exposure to higher temperatures, akin to heatwave events affects reproductive form and function.
Figure 1. Convex hull plot based on PC1 and PC2 scores generated from the analysis of outlines of the bursa copulatrix from 60 females. Colours and lines indicate the three groups in the set: Red = 17°C, Blue = 27°C, and Black = 33°C.

Figure 2. The percentage of pairs copulating in response to male and female developmental temperatures (°C). Females reared at 17°C white bars, 27°C light grey, 33°C dark grey. Numbers above bars represent the number of successful copulations / number of attempted copulations.

Figure 3. The mean (+se) duration of start-to-kick (1st phase), kick-to-end (2nd phase) and total copulation durations in relation to developmental temperature for a) females (17°C n = 8, 27°C n = 29 and 33°C n = 28) and b) males (17°C n = 23, 27°C n = 29 and 33°C n = 30). Horizontal lines represent significant differences following post-hoc Tukey tests: * p < 0.05, ** p < 0.01.

Figure 4. Box and whisker plot of P2 for males from all three treatment groups crossed with 27°C (light grey) and 33°C females (dark grey).
Fig 2

![Chart showing % copulating at different male developmental temperatures. The chart indicates that higher temperatures increase the percentage of males copulating. The data points are as follows:

- 17°C Female: 0/12
- 17°C 27 Female: 23/94
- 17°C 33 Female: 8/51
- 27°C 27 Female: 30/31
- 27°C 33 Female: 28/30
- 33°C 33 Female: 30/30

The chart highlights that 33°C is the optimal temperature for maximal copulating activity.]
Fig 4

![Graph showing developmental temperature vs. R² for female birds at different ages.]

- Male Developmental temperature
- R²

Legend:
- Female 27
- Female 33

Data points and error bars indicate variation in developmental temperatures and R² values across different age groups.
References


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Table 1. Summary of ANOVA on log\(_{10}\) transformed copulation duration data in relation to male and female developmental temperature. 1\(^{st}\) and 2\(^{nd}\) phase of copula refer to the start of copulation to the onset of female kicking behaviour and from the onset of female kicking to the end of copulation respectively.

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) phase of copula</td>
<td>(F_{2,62} = 10.3)</td>
<td>(P &lt; 0.001)</td>
<td>(F_{2,79} = 6.75)</td>
<td>(P = 0.002)</td>
</tr>
<tr>
<td>2(^{nd}) phase of copula</td>
<td>(F_{2,62} = 0.4)</td>
<td>(P = 0.67)</td>
<td>(F_{2,79} = 1.74)</td>
<td>(P = 0.18)</td>
</tr>
<tr>
<td>Total duration</td>
<td>(F_{2,62} = 10.17)</td>
<td>(P &lt; 0.0001)</td>
<td>(F_{2,79} = 5.72)</td>
<td>(P = 0.005)</td>
</tr>
</tbody>
</table>
Highlights

1. A study on ontogenetic temperature and reproductive trait form and function.
2. Used geometric morphometrics to quantify changes in female reproductive tract.
3. Thermal stress during ontogeny alters female reproductive tract morphology.
4. Temperature stress during development alters female reproductive behaviour.
5. Developmental temperature alters the outcome of sperm competition.