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RESEARCH ARTICLE

Reproductive fitness of *Drosophila* is maximised by optimal developmental temperature

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ABSTRACT

Whether the character of developmental plasticity is adaptive or non-adaptive has often been a matter of controversy. Although thermal developmental plasticity has been studied in *Drosophila* for several traits, it is not entirely clear how it affects reproductive fitness. We, therefore, investigated how developmental temperature affects reproductive performance (early fecundity and egg-to-adult viability) of wild-caught *Drosophila melanogaster*. We tested competing hypotheses on the character of developmental thermal plasticity using a full-factorial design with three developmental and adulthood temperatures within the natural thermal range of this species. To account for potential intraspecific differences, we examined flies from tropical (India) and temperate (Slovakia) climate zones. Our results show that flies from both populations raised at an intermediate developmental temperature (25°C) have comparable or higher early fecundity and fertility at all tested adulthood temperatures, while lower (17°C) or higher developmental temperatures (29°C) did not entail any advantage under the tested thermal regimes. Importantly, the superior thermal performance of flies raised at 25°C is apparent even after taking two traits positively associated with reproductive output into account: body size and ovariole number. Thus, in *D. melanogaster*, development at a given temperature does not necessarily provide any advantage in this thermal environment in terms of reproductive fitness. Our findings strongly support the optimal developmental temperature hypothesis, which states that in different thermal environments, the highest fitness is achieved when an organism is raised at its optimal developmental temperature.

KEY WORDS: Developmental plasticity, Acclimation, Thermal performance, Fecundity, Viability, Ovariole number, Body size

INTRODUCTION

In their natural environments, organisms have to cope with spatiotemporal abiotic and biotic changes. Plastic changes in an organism's characteristics (phenotypic plasticity) are considered key features enabling or facilitating survival in varying environments (Schlichting and Smith, 2002; Murren et al., 2015). Phenotypic plasticity is defined as the ability of a single genotype to produce different phenotypes in response to environmental variations (West-Eberhard, 1989). It encompasses morphological, physiological or behavioural changes, including processes such as acclimation or learning (Schlichting and Smith, 2002; Fusco and Minelli, 2010). Plasticity may be a result of direct or indirect effects of the environment on the development of a given trait (Fusco and Minelli, 2010). In cases of direct effects, plasticity is an inevitable, possibly non-adaptive response to environmental stimuli (e.g. the effect of temperature on growth rate) (Stearns, 1989; Fusco and Minelli, 2010). Such phenotypic plasticity is a consequence of an organism's inability to buffer against the impact of environmental changes on physicochemical reactions (Stearns, 1989). By contrast, indirect environmental effects are cues that induce the development of phenotypes that represent adaptive responses to either given or associated environmental conditions (e.g. photoperiod as a cue for the development of seasonal polyphenism) (reviewed in Fusco and Minelli, 2010). Ghalambor et al. (2007) suggest that in genotypes that have never been exposed to a certain environment, the corresponding part of the reaction norm evolves neutrally (non-adaptive plasticity). However, in the natural range of environments, where the reaction norm has been exposed to selection, this part of the reaction norm is adaptive (Ghalambor et al., 2007). Altogether, the character of plasticity – adaptive versus non-adaptive – is often a matter of controversy (reviewed in Via et al., 1995; Gotthard and Nylin, 1995; Ghalambor et al., 2007).

Temperature is probably the major determinant of the spatiotemporal distribution of ectotherms (Clarke, 2003; Bowler and Terblanche, 2008; Angilletta, 2009). Temperature-induced developmental plasticity has influences on thermal tolerance, developmental rate, adult body size and other aspects of adult physiology (reviewed in Johnston and Wilson, 2006). Several different hypotheses have been proposed to explain the nature of thermal developmental plasticity (reviewed in Huey et al., 1999; Wilson and Franklin, 2002). The beneficial acclimation hypothesis states that development at a given temperature confers a fitness advantage within this thermal environment but is disadvantageous at other temperatures. The optimum developmental temperature hypothesis posits that organisms raised at a certain optimal temperature have greater fitness across numerous thermal environments. The hotter-is-better or the cooler-is-better hypothesis proposes that organisms raised in a warmer or colder environment also have better performance at other temperatures. Finally, according to the developmental buffering hypothesis, developmental temperature does not have any effect on adult thermal performance (reviewed in Huey et al., 1999). Among all these diverse concepts, the beneficial acclimation hypothesis has received by far the most attention (e.g. Gibert et al., 2001; Gilchrist and Huey, 2001; Huey et al., 1995; Nunney and Cheung, 1997; Frazier et al., 2008). However, numerous studies that tested this hypothesis for various traits in different species did not support its general validity (reviewed in Huey et al., 1999). Altogether, this indicates that the nature of developmental thermal plasticity might vary among individual traits and/or among species.

Here, we analysed and tested competing hypotheses on the nature of thermal developmental plasticity in the fruit fly *Drosophila*

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melanogaster. Although thermal developmental plasticity has been studied in *Drosophila* for several traits (e.g. Zwaan et al., 1992; Zamudio et al., 1995; Gibert et al., 2001), the effect of developmental temperature on reproductive fitness has not been systematically investigated. In order to discriminate between opposing hypotheses on thermal developmental plasticity, we employed a full-factorial experimental design (Huey et al., 1999; Wilson and Franklin, 2002). Specifically, we examined the effects of three developmental temperatures (17, 25 and 29°C) on early fecundity and egg-to-adult viability at three adulthood temperatures. The three experimental temperatures are within the natural thermal range of *D. melanogaster* (Pétavy et al., 1997) and are not considered extreme or pathological (Gibert et al., 2001). Because of a very short lifespan in the natural environment due to high extrinsic mortality (Rosewell and Shorrocks, 1987) and a strong positive correlation between early and lifetime fecundity (Klepsatel et al., 2013b; Nguyen and Moehring, 2015), the early reproductive performance may be considered a good estimator of individual reproductive fitness (Klepsatel et al., 2013b). Moreover, to account for potential intraspecific differences in the nature of temperature-induced developmental plasticity, we used two populations originating from markedly different thermal environments, i.e. from temperate (Slovakia) and tropical (India) climate areas. Finally, we analysed how variations in body size and ovariole number affect the thermal performance of reproduction. These traits are known to be significantly affected by developmental temperature and to have a positive impact on fecundity (e.g. Robertson, 1957; Tantawy and Rakha, 1964; David, 1970; Lefranc and Bundgaard, 2000; Klepsatel et al., 2013b). Based on the aforementioned hypotheses on the nature of thermal developmental plasticity, we expected that under the beneficial acclimation hypothesis, flies that had developed at a given temperature should show the highest fitness at this temperature. In contrast, the finding that development at a distinct (optimal) temperature leads to superior performance at all experimental temperatures would provide strong support for the optimal developmental temperature hypothesis. Alternatively, under the hotter-is-better hypothesis, the highest reproductive performance should be found in flies that had developed at 29°C; under the cooler-is-better hypothesis, the highest fitness should be achieved in flies

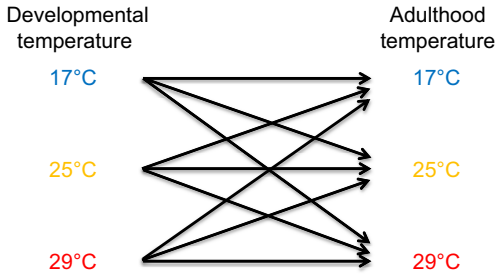

Fig. 1. Full factorial experimental design with three developmental and three adulthood temperatures.

Table 1. Multi-way analyses of variance (ANOVA) testing the effects of population, developmental temperature, adulthood temperature and their interactions on early fecundity, egg-to-adult viability at day 10, early fecundity per body size and early fecundity per ovariole

Trait	Source of variation	d.f.	SSQ	*F*-ratio	*P*-value
Early fecundity	Population	1	3,011,329	140.38	<0.0001
	Developmental temperature	2	1,685,365	39.28	<0.0001
	Adulthood temperature	2	20,017,971	466.58	<0.0001
	Population×developmental temperature	2	5169	0.12	0.887
	Population×adulthood temperature	2	257,132	5.99	0.003
	Developmental temperature×adulthood temperature	4	350,956	4.09	0.003
	Population×developmental temperature×adulthood temperature	4	220,390	2.57	0.038
	Error	400	8,580,803	–	–
Egg-to-adult viability (day 10)	Population	1	0.27	3.94	0.048
	Developmental temperature	2	3.74	26.84	<0.0001
	Adulthood temperature	2	17.00	121.91	<0.0001
	Population×developmental temperature	2	1.70	12.23	<0.0001
	Population×adulthood temperature	2	0.56	4.00	0.019
	Developmental temperature×adulthood temperature	4	2.19	7.86	<0.0001
	Population×developmental temperature×adulthood temperature	4	1.15	4.11	0.003
	Error	367	25.59	–	–
Early fecundity per body size	Population	1	1,993,556.3	63.54	<0.0001
	Developmental temperature	2	2,756,035.8	43.92	<0.0001
	Adulthood temperature	2	5,913,955.6	94.24	<0.0001
	Population×developmental temperature	2	5301.2	0.08	0.919
	Population×adulthood temperature	2	71,548.1	1.14	0.321
	Developmental temperature×adulthood temperature	4	843,934.1	6.72	<0.0001
	Population×developmental temperature×adulthood temperature	4	133,888.5	1.07	0.373
	Error	400	12,550,294	–	–
Early fecundity per ovariole	Population	1	121.7	7.66	0.006
	Developmental temperature	2	601.1	18.92	<0.0001
	Adulthood temperature	2	3348.9	105.40	<0.0001
	Population×developmental temperature	2	115.3	3.63	0.027
	Population×adulthood temperature	2	76.9	2.42	0.090
	Developmental temperature×adulthood temperature	4	415.4	6.54	<0.0001
	Population×developmental temperature×adulthood temperature	4	162.5	2.56	0.038
	Error	399	6338.8	–	–

Early fecundity was measured as the mean cumulative number of eggs laid during the first 10 days after eclosion, and is also presented per body size (thorax length^3) and per ovariole. Egg-to-adult viability was measured as the proportion of eggs laid at day 10 that developed into adults. d.f., degrees of freedom; SSQ, the sum of squares for each source of variation.

Journal of Experimental Biology (2019) 222, jeb202184. doi:10.1242/jeb.202184

that had developed at 17°C. Finally, under the developmental buffering hypothesis, we should not be able to detect any significant effect of developmental temperature on reproductive performance. Our experiments showed that flies from both populations that developed at 25°C have comparable or higher reproductive fitness at all tested temperatures. This effect was apparent even after taking differences in body size and ovariole number into account. Taken together, our findings are not consistent with the beneficial acclimation hypothesis, but instead provide solid support for the optimal developmental temperature hypothesis.

MATERIALS AND METHODS

Fly populations

Two outbred wild-caught populations of *D. melanogaster* Meigen were used that originated from (1) Slovakia (Bratislava, 48.21°N, 17.15°E; collected by L' Vidlička in October 2017) and (2) India (Mysore, Karnataka, 12.30°N, 76.64°E; collected by the members of the *Drosophila* stock centre at the University of Mysore in July 2017). Both populations were established from at least 300 freshly collected females and males that were kept as mass-bred populations at a population size of ∼1500–2000 adults for 3–6 months prior to the experiments. All flies were maintained in vials on a standard *Drosophila* medium (6 g agar, 50 g yeast, 50 g sucrose, 70 g maize flour, 5.12 ml propionic acid and 1.3 g methyl paraben per 1 litre of medium) at 25°C (12 h:12 h light:dark, 60% humidity) with overlapping generations (generation time ∼3 weeks).

Fecundity and viability

To test the effect of developmental temperature on adult fitness, we used a full-factorial design with three 'developmental' and three 'adulthood' temperatures (Fig. 1). Two-week-old parental flies (∼100 individuals) laid eggs into vials during a 3 h period; vials with a medium egg density (∼150 eggs per 68 ml vial; any superfluous eggs were removed) were randomly distributed between the 17, 25 and 29°C groups (12 h:12 h light:dark, 60% humidity). Freshly eclosed flies (collected within 12 h of eclosion) from each temperature and each of the two populations were randomly placed into three adulthood temperatures, in order to establish nine different groups for each population. Each group consisted of 20–24 females, which were placed individually with two males (reared at the same temperature as the females) into vials (46 ml) containing a standard *Drosophila* medium with ca. 10 mg of active dry yeast sprinkled on top of the food. Flies kept at 17°C were transferred every 48 h; flies at 25 and 29°C were transferred every 24 h. The number of eggs laid during this period was counted under a stereomicroscope. Fecundity was measured as the cumulative number of eggs laid by a single female during either the first 10 days (at 25 and 29°C) or 20 days (at 17°C). After counting, vials with eggs laid on the 5th and the 10th day at 25 and 29°C or on the 10th and the 20th day at 17°C were returned to a given adulthood temperature in order to determine the egg-to-adult viability (measured as the proportion of eclosed flies). At the end of the experiment, the thorax length of all females was measured and the number of ovarioles was counted after dissection (see below). Data

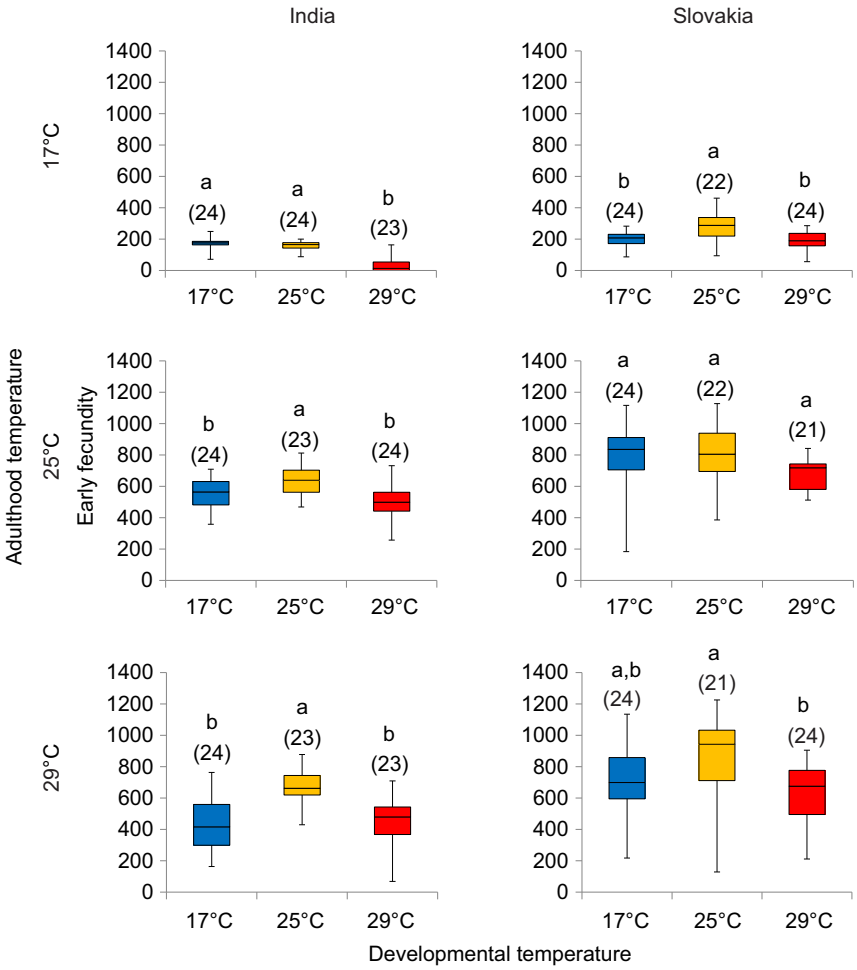

Fig. 2. Effect of developmental temperature on early fecundity at three adulthood temperatures. Early fecundity was measured as the cumulative number of eggs laid during the first 10 days after eclosion. Flies from both populations that developed at intermediate temperature (25°C) show early fecundity at all three experimental adulthood temperatures that is equal to or higher than that of cold- or warm-reared flies. Sample size is reported in parentheses. Data for each population and adulthood temperature were compared by one-way ANOVA followed by Tukey's HSD test (α=0.05). Groups with the same letters are not significantly different from each other. Box plots display minimum, first quartile, median, third quartile and maximum value. For further statistical analyses, see Table 1 and Tables S1 and S2.

Journal of Experimental Biology (2019) 222, jeb202184. doi:10.1242/jeb.202184

on females that escaped or died during the experiment were excluded from all analyses.

Morphological traits

Thorax length was measured under a Leica M205C stereomicroscope by use of a Leica DFC295 digital camera and Leica application software. Thorax length was measured from the base of the most anterior humeral bristle to the posterior tip of the scutellum on the left side of the fly (French et al., 1998). Ovaries were dissected in tap water and the numbers of ovarioles counted under a stereomicroscope (Zeiss Stemi 2000). Ovariole number is expressed as the sum of ovarioles from both ovaries.

Statistical analysis

We first analysed the effects of population, developmental and adulthood temperature on early fecundity by using full-factorial, multi-way fixed-effects analysis of variance (ANOVA). Next, we analysed the effects of population and developmental temperature on early fecundity at different temperatures using full-factorial, two-way fixed-effects ANOVA. Third, we examined the effect of developmental temperature on early fecundity at different temperatures separately for each population by one-way ANOVA with Tukey's HSD *post hoc* test. A similar approach was used for analysing viability. To account for variation in thorax length and ovariole number, we analysed early fecundity by analysis of covariance (ANCOVA), with developmental temperature as the fixed factor and thorax length or ovariole number as the covariate, followed by Tukey's HSD *post hoc* test. These analyses were performed separately for each population and adulthood temperature. Fecundity per body size and fecundity per ovariole for a given population and at a given adulthood temperature were both examined by one-way ANOVA with developmental temperature as the fixed factor, followed by Tukey's HSD *post hoc* test. All analyses were performed with JMP v.14.1.0 (SAS, Raleigh, NC, USA) and PAST 3.11 (https://folk.uio.no/ohammer/past/) software.

RESULTS

Developmental temperature has a significant effect on early fecundity

Global analysis (multi-way ANOVA) of the influence of three different factors on early fecundity showed that all three variables (population, developmental temperature and adulthood temperature) and three of their interactions (population×adulthood temperature, developmental temperature×adulthood temperature and population×developmental temperature×adulthood temperature) had

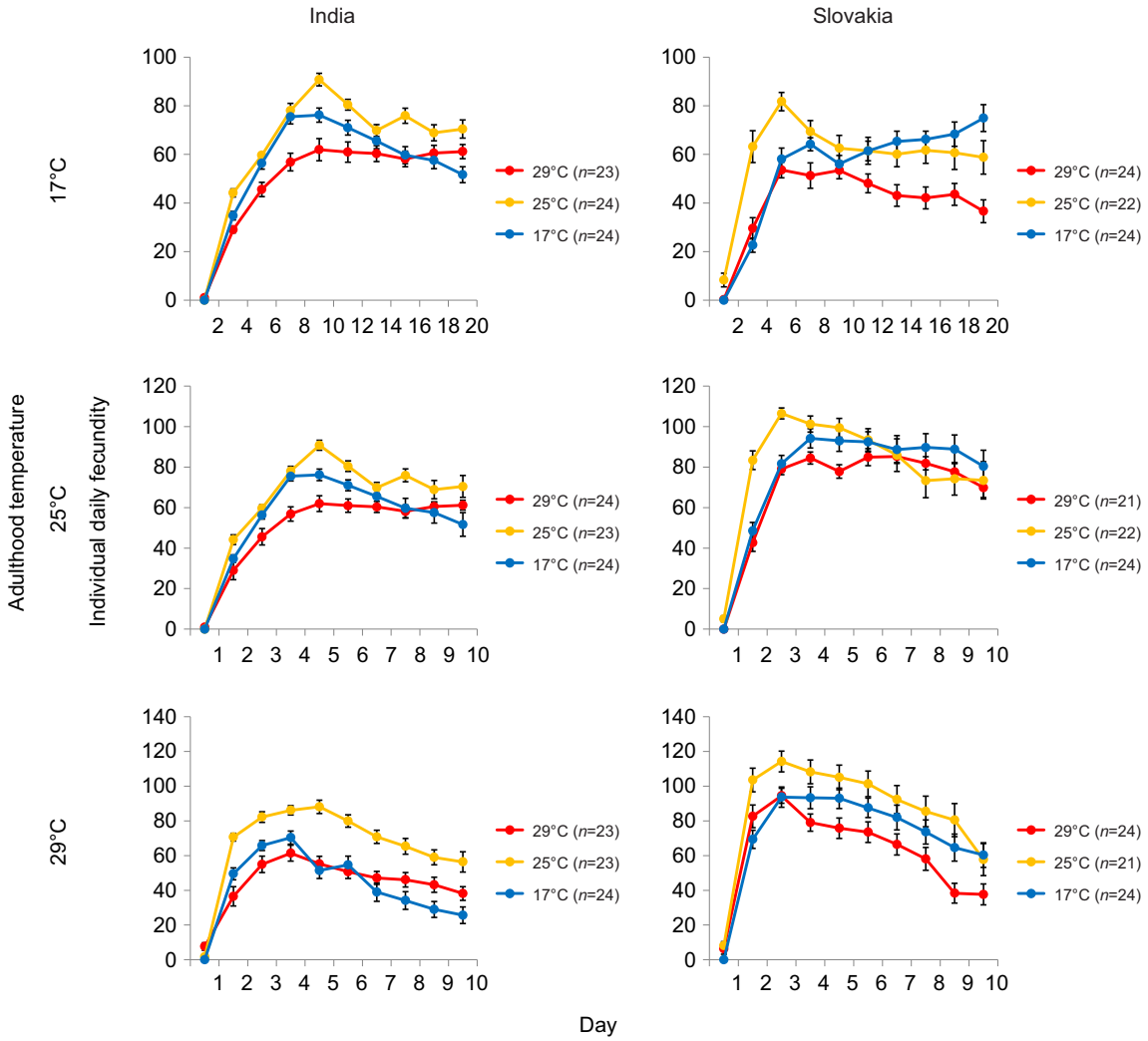

Fig. 3. Mean (±s.e.m.) individual daily fecundity at three adulthood temperatures. Different colours depict cohorts of flies that developed at different temperatures. Sample size (*n*) is reported in parentheses.

Journal of Experimental Biology (2019) 222, jeb202184. doi:10.1242/jeb.202184

a significant effect (Table 1). Early fecundity was lowest at 17°C and highest at 25°C. Overall, flies from Slovakia showed higher fecundity than flies from India at all experimental temperatures (Figs 2 and 3).

When examined at the low adulthood temperature (17°C), the two experimental populations differed significantly with regard to the effect of developmental temperature on fecundity (Table S1). In the Indian population, early fecundity (measured over the first 10 days) at 17°C was significantly higher in individuals that had developed at 17 and 25°C than in flies that were reared at 29°C (Figs 2 and 3; Table S2). In contrast, flies from Slovakia that had developed at 25°C showed significantly higher fecundity than flies that had developed at 17 or 29°C (Figs 2 and 3; Table S2). As oogenesis is substantially slower at lower temperatures, we continued measuring individual fecundity at 17°C until day 20 post-eclosion. In contrast to early fecundity measured over the first 10 days, we did not detect any significant intraspecific difference in the effect of developmental temperature on cumulative fecundity measured over the first 20 days (Table S2). In both populations, flies that had developed at 17 or 25°C showed a similar reproductive output, which was higher than that of flies that had developed at 29°C (Fig. S1A, Table S2).

At the intermediate adulthood temperature (25°C), both population and developmental temperature had a significant impact on fecundity; however, there was no significant interaction between population and developmental temperature (Table S1). Individuals that had developed at 25°C laid more eggs at this temperature than flies that had developed at low or high temperatures; however, this effect was statistically significant only in the Indian population (Fig. 2; Table S2).

Population and developmental temperature both also had significant effects on early fecundity at high temperature (29°C) (Table S1). In both populations, the early fecundity examined at 29°C was highest in the flies that had developed at intermediate temperature (25°C) (Figs 2 and 3; Table S2).

Altogether, flies from both populations that had developed at the intermediate temperature (25°C) had an equal or higher early fecundity under all three experimental temperatures when compared with the cold- or warm-reared flies.

Developmental temperature of parents affects egg-to-adult viability

Similar to fecundity, egg-to-adult viability was significantly affected by population, developmental and adulthood temperature,

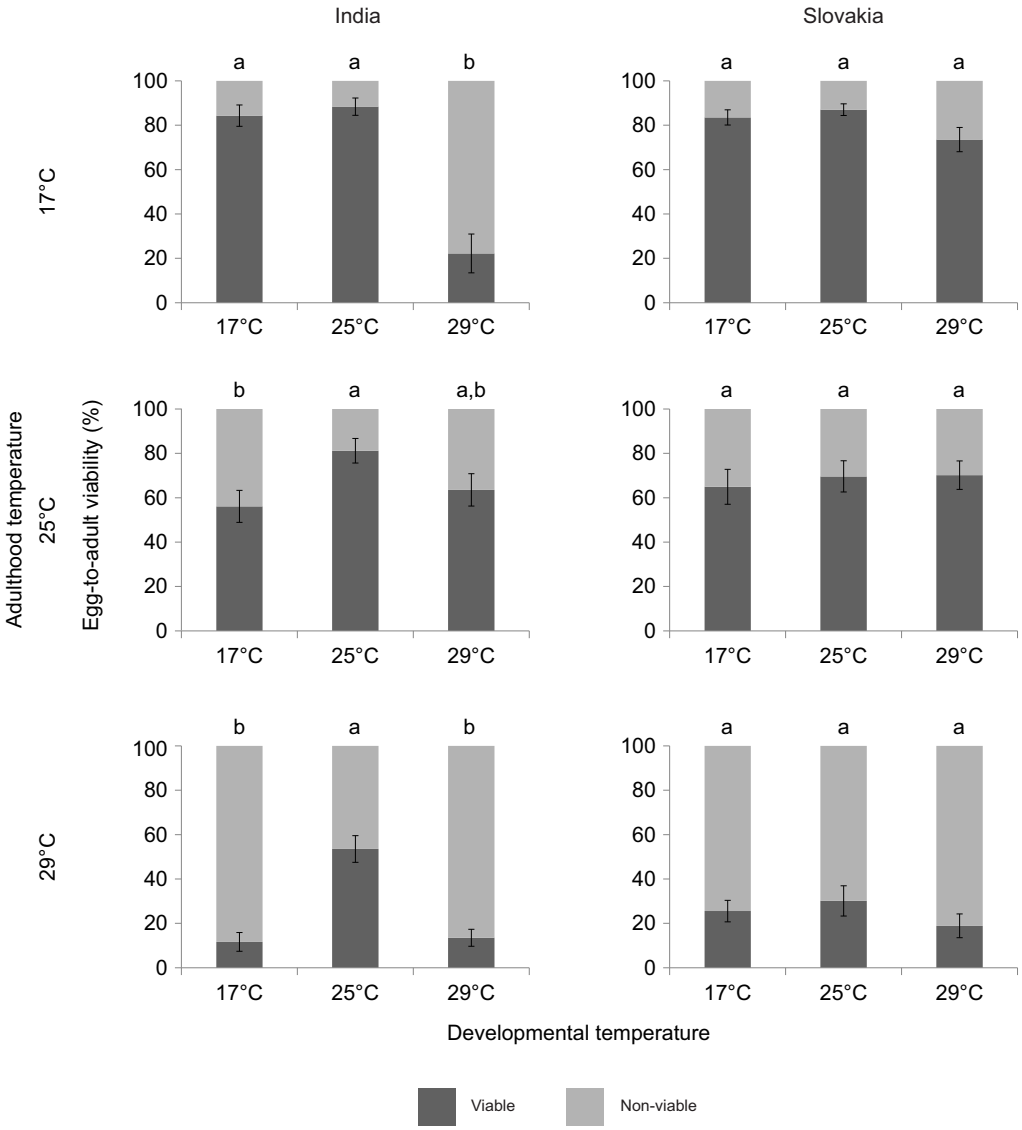

Fig. 4. Effect of developmental temperature on egg-to-adult viability. Viability was measured as the number of eggs laid at day 10 after eclosion. Data for each population and adulthood temperature were compared by one-way ANOVA followed by Tukey's HSD test (α=0.05). Groups with the same letters are not significantly different from each other. Error bars represent s.e.m. For additional statistical analyses, see Table 1 and Table S3.

Journal of Experimental Biology (2019) 222, jeb202184. doi:10.1242/jeb.202184

as well as by their interactions (Table 1; Table S3). In general, the viability of the progeny decreased with age and on average also with adulthood temperature (Fig. 4; Table S2). Developmental temperature of the parents had a significant effect on the egg-to-adult viability of flies from India (Fig. 4; Fig. S2). Eggs laid by parents that had developed at 25°C were on average more viable at all three experimental temperatures than eggs laid by females that had developed at 17 or 29°C (Fig. 4; Fig. S2). This effect of parental developmental temperature on egg-to-adult viability was absent or much less pronounced in the fly population from Slovakia when compared with that from India (Fig. 4; Fig. S2).

Developmental temperature also affects early fecundity independent of body size

It is a well-known phenomenon that body size of ectotherms decreases with increasing developmental temperature, the so-called temperature–size rule (e.g. Angilletta and Dunham, 2003). The inverse relationship between body size (measured here as thorax length) and developmental temperature was supported by our experimental data (Fig. S3A). Therefore, we additionally analysed our data on fecundity with regard to observed variations in thorax length (Fig. 5). The results of these analyses (ANCOVA) were essentially similar to our previous results on fecundity, i.e. flies from the intermediate developmental temperature (25°C) group showed a thermal reproductive performance that was similar to or higher than that of flies from the lower or higher developmental temperature groups (Tables S4 and S5).

In addition, we examined the effect of developmental temperature on relative fecundity, defined as the number of eggs per unit body size (thorax length^3). Consistent with our previous analysis, developmental and adulthood temperature had qualitatively similar effects on the relative as well as on the absolute fecundity (Fig. 6, Table 1; Fig. S1B, Table S2). Overall, these results indicate that the observed changes in the early fecundity of flies that had developed at different temperatures cannot be explained solely by the differences in body size.

Developmental temperature, ovariole number and early fecundity

Finally, we analysed early fecundity in relation to variations in ovariole number (Fig. 7). Overall, flies from tropical India had on average fewer ovarioles than flies from temperate Slovakia (Fig. S3B); the ovariole number tended to be highest in flies that had developed at 25°C and lowest in flies that had been reared at 29°C (Fig. S3B). Next, we analysed early fecundity with ovariole number as a covariate (ANCOVA) (Table S4) and also examined the fecundity per ovariole (calculated as the number of eggs per ovariole) (Fig. 8, Table 1; Fig. S1C, Table S2). Both analyses yielded qualitatively similar results to the analyses of absolute fecundity, i.e. flies that had developed at the intermediate temperature (25°C) had an equal or higher reproductive

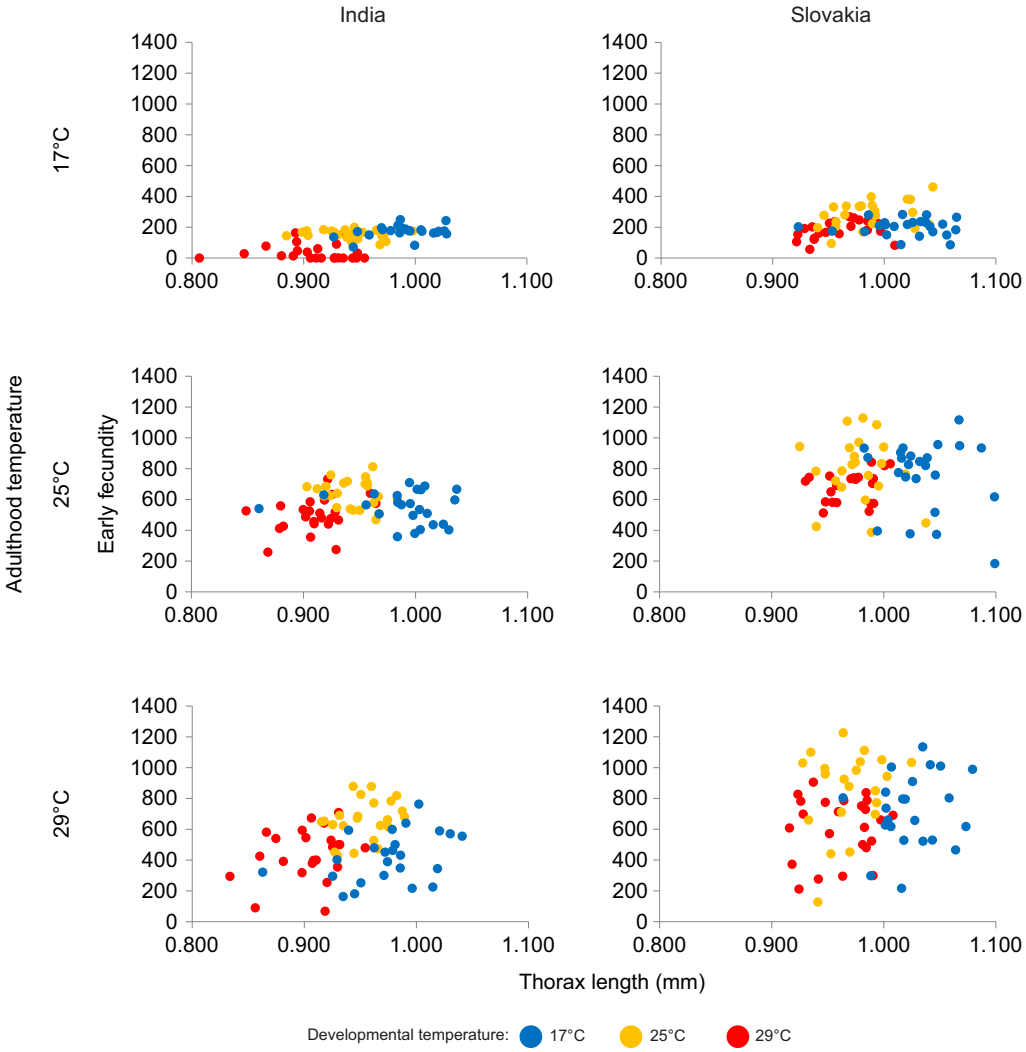

Fig. 5. The relationship between thorax length and early fecundity is affected by developmental temperature. Early fecundity was measured as for Fig. 2. Each dot represents data from an individual female.

Journal of Experimental Biology (2019) 222, jeb202184. doi:10.1242/jeb.202184

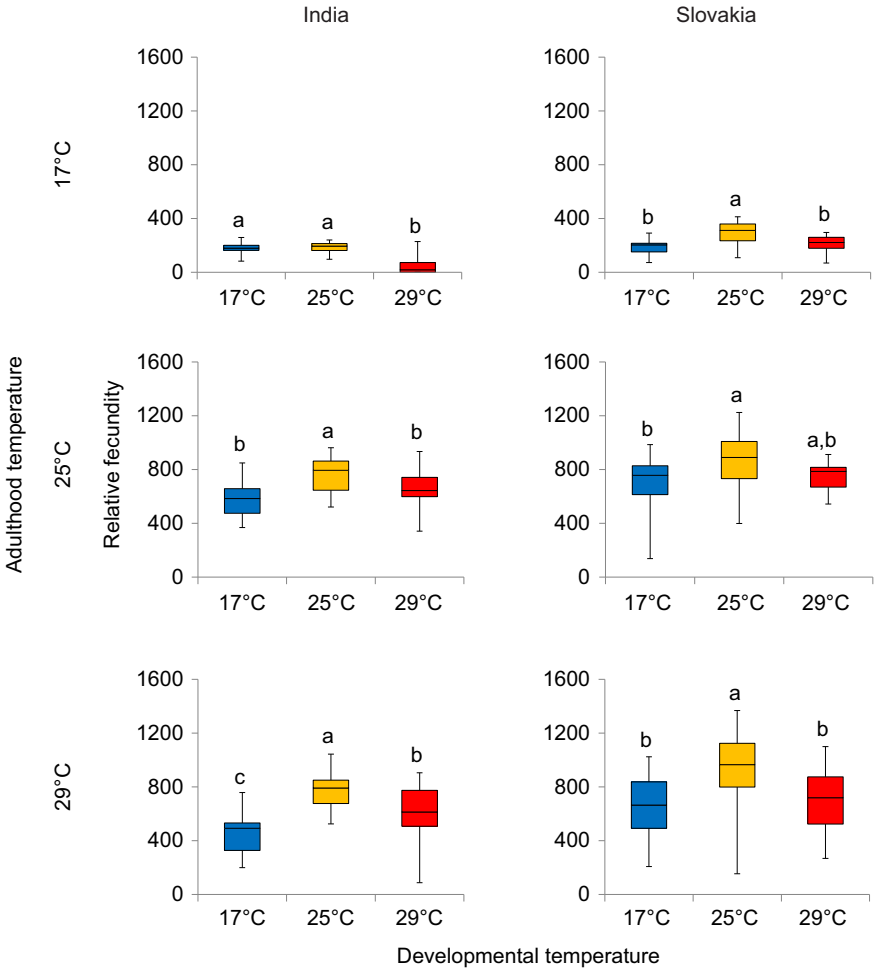

Fig. 6. Effect of developmental temperature on relative fecundity. Relative fecundity was measured as the cumulative number of eggs laid during the first 10 days after eclosion per unit body size (thorax length^3). Flies raised at 25°C have on average higher relative fecundity than flies reared at lower or higher temperatures. Sample sizes are the same as reported in Fig. 2. Data for each population and adulthood temperature were compared by one-way ANOVA followed by Tukey's HSD test (α=0.05). Groups with the same letters are not significantly different from each other. Box plots display minimum, first quartile, median, third quartile and maximum value. For further statistical analyses, see Table 1 and Table S2.

performance at the temperatures examined when compared with the cold- or warm-reared flies.

DISCUSSION

In the present study, we explored how developmental temperature affects reproductive fitness. By employing a full-factorial design with more than two thermal regimes, this study is the first to systematically investigate the effect of developmental temperature on thermal reproductive performance in insects. Contrary to the developmental acclimation hypothesis, we found that development at a given temperature does not necessarily lead to superior reproductive performance in this thermal environment. Flies raised at the intermediate developmental temperature (25°C) had a similar or higher reproductive fitness in comparison to flies that had developed at lower or higher temperatures. These results strongly support the optimal developmental temperature hypothesis. Although we detected some intraspecific differences in the effect of developmental temperature on early fecundity, a higher reproductive output of flies raised at the intermediate temperature was observed in both populations examined.

Cohet and David (1978) examined early fecundity (the first 10 days after eclosion) and lifetime egg production at 25°C in *Drosophila* reared at 10 different temperatures ranging from 12 to 32°C. They found that both early and total fecundity were maximised if the females had developed at 21 and 25°C, which is consistent with our results on the flies from India. However, we did not detect any significant differences between the flies from Slovakia that had developed at 17 versus 25°C with regard to early fecundity at 25°C. We assume that this result may reflect potential intraspecific differences. In contrast to our study, Nunney and Cheung (1997) found evidence for adaptive acclimation effects of rearing temperature on early productivity (number of offspring) in *Drosophila*. These authors used a full-factorial design with two rearing temperatures (18 and 25°C). In their study, early productivity was higher when developmental and adult temperatures were identical (Nunney and Cheung, 1997). The fact that our results are not consistent with this study can be explained by major differences in experimental design. Fecundity shows a peak at 25°C within the first 3–4 days of adulthood (Klepsatel et al., 2013b). However, in the study of Nunney and Cheung (1997), productivity was measured for days 7–17 after eclosion at 18°C and for days 5–12 after eclosion at 25°C. Thus, the very early fecundity/productivity was not taken into account. Another important difference is that, in order to examine the full reproductive potential, we supplemented our experimental food with yeast. Yeasts are a natural source of proteins (Skorupa et al., 2008) and sterols for saprophytic *D. melanogaster* (Bos et al., 1976); their absence strongly reduces fecundity (Robertson and Sang, 1944). Thus, in our experiments, daily fecundity and productivity were several times higher than in the study by Nunney and Cheung (1997), which may facilitate detection of potential differences in reproductive performance. It is also noteworthy that Gilchrist and Huey (2001) tested the effect of parental temperature on offspring fitness. They found that parents reared at higher temperature produced progeny with higher fitness, independent of their thermal

Journal of Experimental Biology (2019) 222, jeb202184. doi:10.1242/jeb.202184

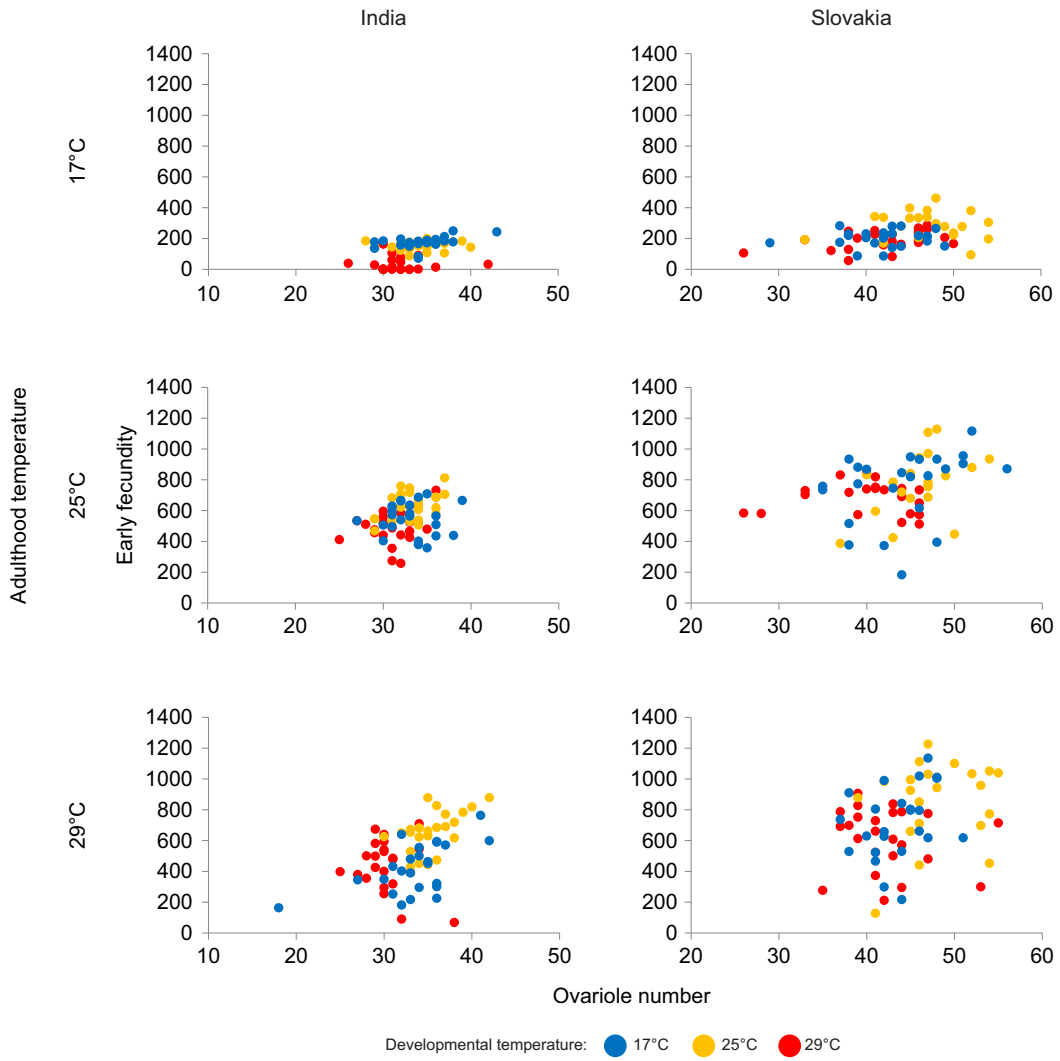

Fig. 7. The relationship between ovariole number and early fecundity at three adulthood temperatures in flies that developed at different temperatures. Early fecundity was measured as for Fig. 2. Each dot represents data from an individual female.

environment. This effect was caused by faster development of the offspring (Gilchrist and Huey, 2001).

Consistent with our results, numerous studies in *Drosophila* on other traits provide evidence in support of the optimal developmental temperature hypothesis. For example, Zamudio et al. (1995) studied the effect of developmental temperature (18 versus 25°C) on male territorial success at low (18°C) and high temperature (27°C). Despite their smaller body size, males raised at 25°C were more successful at both temperatures than males that had developed at 18°C (Zamudio et al., 1995). Similarly, walking speed of *D. melanogaster* tested at three different temperatures (18, 25 and 29°C) was generally highest if flies had developed at intermediate temperature and lowest if they had been reared at high temperature, although intraspecific differences were also apparent (Gibert et al., 2001). Finally, Zwaan et al. (1992) examined lifespan in a full-factorial design using three temperatures (20, 25 and 29°C). Males and females reared at the intermediate temperature had the longest lifespan, whereas flies reared at the lowest developmental temperature had the shortest lifespan. However, beneficial developmental plasticity has also been confirmed in *Drosophila*. For instance, Kristensen et al. (2008) found that flies that had developed at low temperature (15°C) were substantially better at finding a food resource in a cold environment; conversely, flies reared at 25°C were better at finding food at higher temperatures. Frazier et al. (2008) consistently showed that cold-reared flies (at 15°C) had improved flight capability at low temperature (14°C) as a result of increased wing area and wing length. However, at 18°C, flies reared at different temperatures (15, 23, 28°C) had similar flight performance. Overall, these findings suggest that the nature of thermal developmental plasticity is most likely trait specific.

One of the interesting effects of temperature on the majority of examined ectotherms is an inverse relationship between body size and developmental temperature, the so-called temperature–size rule (Atkinson, 1994). Although multiple adaptive and non-adaptive hypotheses explaining this phenomenon have been proposed (e.g. Berrigan and Charnov, 1994; Atkinson and Sibly, 1997; Angilletta and Dunham, 2003; Angilletta et al., 2004; Walters and Hassall, 2006; Arendt, 2011), the answer to the fundamental question of why ectotherms are usually larger in colder environments remains unclear (Klok and Harrison, 2013). In general, a positive relationship between body size and fitness has been documented in many species (reviewed in Chown and Gaston, 2010). Larger body size correlates with higher fecundity in females and higher mating success in males (e.g. Honěk, 1993; Andersson, 1994).

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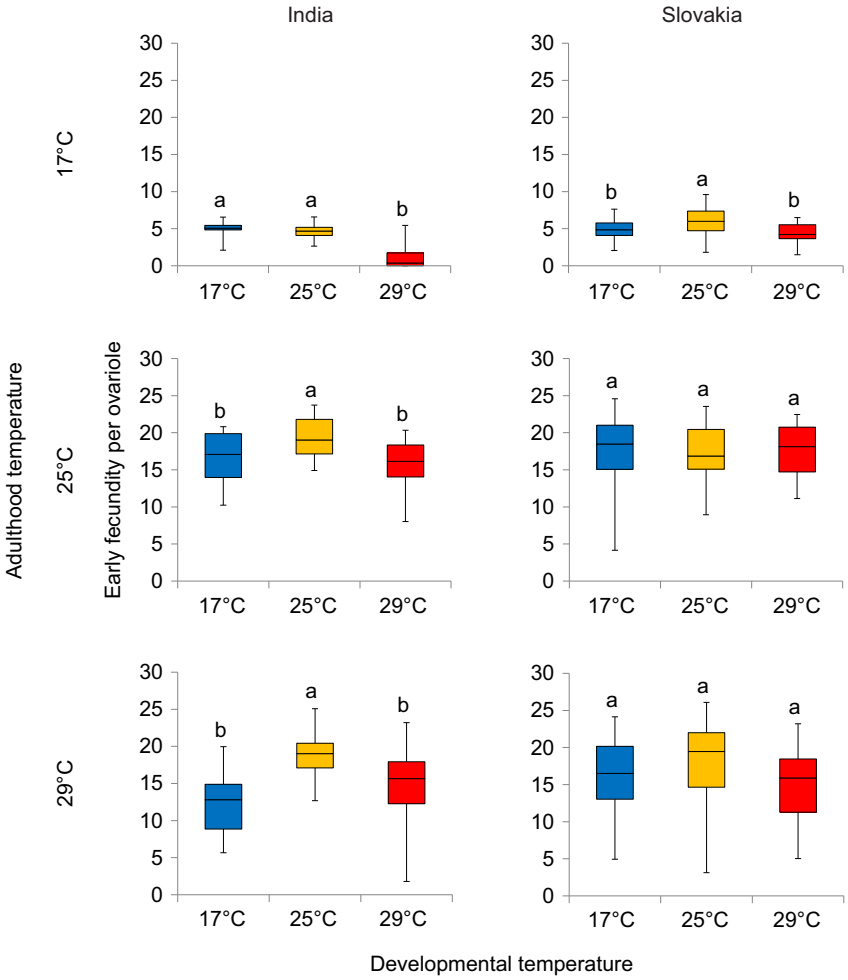

Fig. 8. Effect of developmental temperature on early fecundity per ovariole. Early fecundity was measured as for Fig. 2 and is given per ovariole. Flies from both populations raised at the intermediate temperature (25°C) have comparable or higher fecundity per ovariole compared with the cold- or warm-reared flies. Sample sizes are the same as reported in Fig. 2. Data for each population and adulthood temperature were compared by one-way ANOVA followed by Tukey's HSD test (α=0.05). Groups with the same letters are not significantly different from each other. Box plots display minimum, first quartile, median, third quartile and maximum value. For additional statistical analyses, see Table 1 and Table S2.

Nevertheless, it is still unclear whether the temperature–size rule is an adaptation or an inevitable response driven by physiological constraints (reviewed in Atkinson and Sibly, 1997; Angilletta et al., 2004). In our experiments, the cold-reared flies had – despite their larger body size – either comparable or lower reproductive performance than smaller flies that had developed at 25°C. After correcting for body size, flies from the low developmental temperature group were even less fecund than flies from the intermediate developmental temperature group. This suggests that an increase in body size, as a plastic response to lower developmental temperature, might not primarily be adaptive in relation to early fecundity.

Ovariole number is another trait that has a direct positive effect on fecundity (David, 1970; Klepsatel et al., 2013b). Consistent with the results from previous studies (e.g. Capy et al., 1993; Schmidt et al., 2005; Klepsatel et al., 2014; Rajpurohit et al., 2017), flies from tropical India had on average fewer ovarioles than flies from temperate Slovakia. Although ovariole number is positively correlated with body size, this holds true only for flies that develop at the same temperature (Klepsatel et al., 2013a). Unlike for body size, the optimal developmental temperature for ovariole number in *D. melanogaster* is 23–24°C, i.e. flies that develop at lower temperatures have fewer ovarioles despite their larger body size (Klepsatel et al., 2013a). Importantly, our analysis showed that the larger cold-reared flies had either similar or lower fecundity per ovariole when compared with flies that had developed at the intermediate temperature. As flies reared at 25°C had more ovarioles than flies that had developed at either 17 or 29°C, the observed differences in their reproductive performance might just reflect the differences in ovariole number. However, even when considering the fecundity per ovariole, flies from India that had developed at the intermediate temperature tended to have higher egg production per ovariole than flies reared at the low or high temperatures. Thus, the differences in ovariole number alone cannot explain the observed differences in reproductive performance.

Woods and Harrison (2002) have argued that a rejection of the beneficial acclimation hypothesis can be a consequence of comparing the performances of organisms that had developed in non-optimal versus optimal environments. Prolonged exposure to non-optimal conditions during development may have a long-lasting detrimental effect which negatively affects adult performance (Woods and Harrison, 2002). Although the three experimental temperatures we have chosen are within the natural thermal range of *D. melanogaster* (Pétavy et al., 1997), the observation that all flies had reduced fertility at 29°C, probably due to high-temperature-induced male sterility (reviewed in David et al., 2005), indeed suggests that development within this thermal environment is stressful, leading to long-term physiological consequences that reduce fitness (cf. Chakir et al., 2002). Considering development at 17°C, this particular temperature is already outside the natural thermal range of the flies from India. Thus, development at this temperature could be stressful for these flies. However, we did not reveal any substantial qualitative intraspecific differences in the reproductive performance at 17°C

that would clearly indicate that the flies from tropical India were more affected by the low developmental temperature than the flies collected in temperate Slovakia, which are most likely well adapted to such low temperatures. Thus, it seems improbable that long-term exposure to 17°C could have been detrimental.

Importantly, the thermal tolerance of adult *Drosophila* is significantly influenced by developmental temperature (e.g. Gibert and Huey, 2001; Ayrinhac et al., 2004; Overgaard et al., 2008; Colinet and Hoffmann, 2012; Kellermann et al., 2017; Schou et al., 2017a,b). Cold-reared flies are more resistant to cold than are warm-reared flies, which, by contrast, are more tolerant to heat (Schou et al., 2017a). Moreover, this effect of developmental temperature on thermal tolerance may explain the observed differences between flies reared at 17 and 25°C with regard to their reproductive performance at 29°C. Whilst a prolonged exposure to 29°C may not be particularly challenging for the flies reared at the intermediate developmental temperature, flies that had developed at low temperature may in fact be negatively affected by higher temperature, given their presumably lower heat resistance.

In summary, our results on the effect of developmental temperature on reproductive performance and fitness at different temperatures provide strong and solid support for the optimal developmental temperature hypothesis. We assume that the effect of developmental temperature on reproductive performance results from a complex interplay of numerous factors that are determined by the thermal environment during development, such as ovariole number, body size, thermal tolerance or the degree of thermal damage.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: P.K., M.G.; Methodology: P.K., M.G.; Formal analysis: P.K., T.N.G.; Investigation: P.K.; Writing - original draft: P.K., H.D.; Writing - review & editing: P.K., T.N.G., H.D., M.G.

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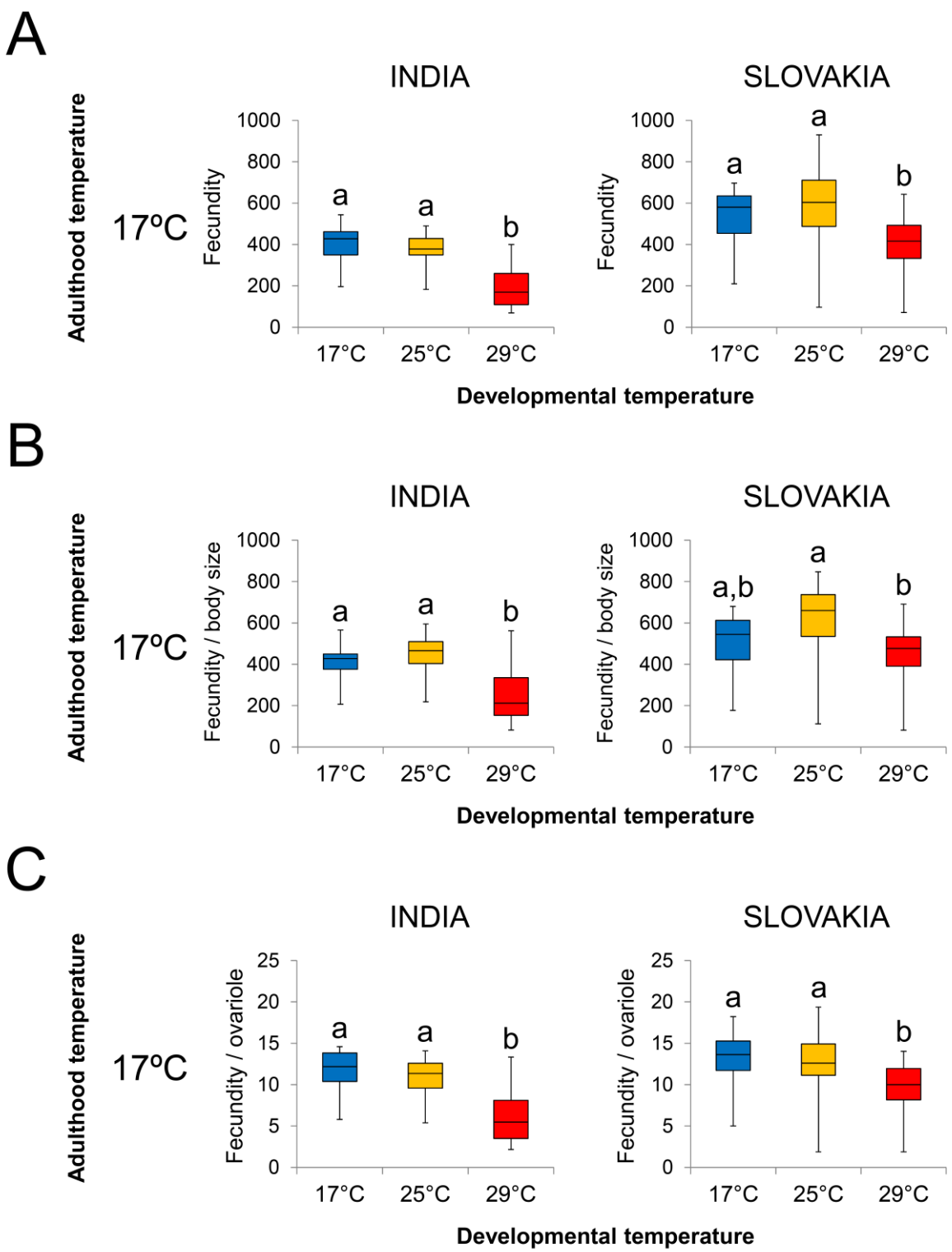
A
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SLOVAKIA
Adulthood temperature
17°C
Fecundity
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b
17°C
25°C
29°C
Developmental temperature
B
INDIA
SLOVAKIA
Adulthood temperature
17°C
Fecundity / body size
a
a
b
a,b
a
b
Developmental temperature
C
INDIA
SLOVAKIA
Adulthood temperature
17°C
Fecundity / ovariole
25
20
15
10
5
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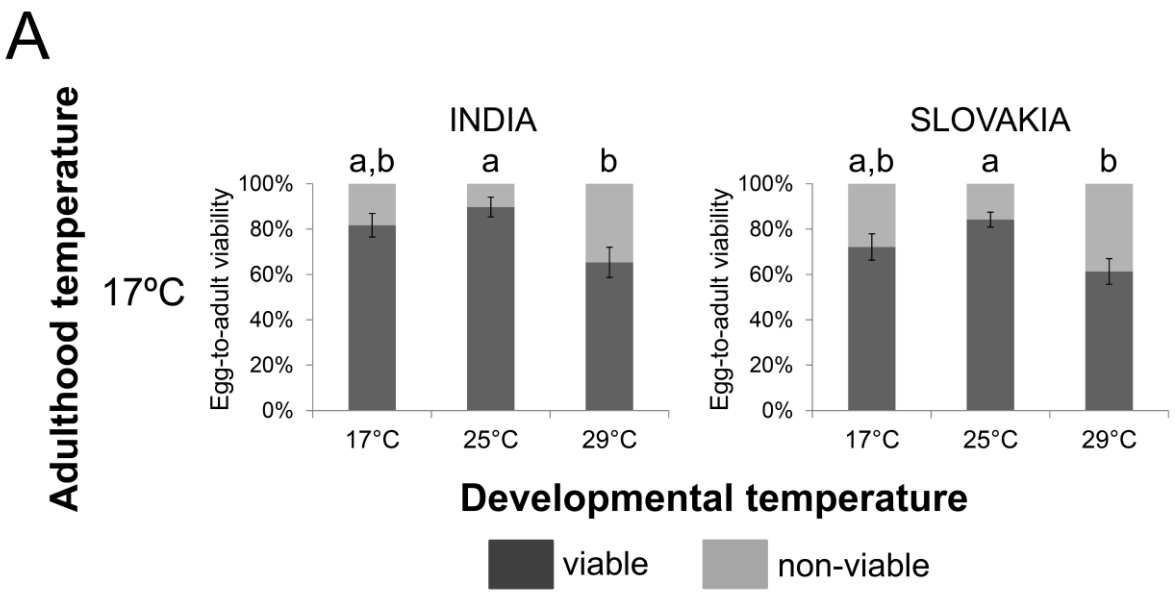
a
a
b
a
a
b
Developmental temperature

Figure S1. Effect of developmental temperature on early fecundity (cumulative number of eggs during the first twenty days after eclosion) (**A**), relative fecundity (cumulative number of eggs laid during the first twenty days after eclosion per unit body size (thorax length^3)) (**B**), and early fecundity per ovariole (cumulative number of eggs laid during the first twenty days after eclosion per ovariole) at 17°C (**C**). Data for each population, adulthood temperature and trait were compared by one-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$). Groups with the same letters are not significantly different from each other. Box plots display minimum, first quartile, median, third quartile, and maximum value. For statistical analyses see Table S1, S2.


A
Adulthood temperature
17ºC
INDIA
SLOVAKIA
a,b
a
b
Egg-to-adult viability
100%
80%
60%
40%
20%
0%
17°C
25°C
29°C
Developmental temperature
viable
non-viable

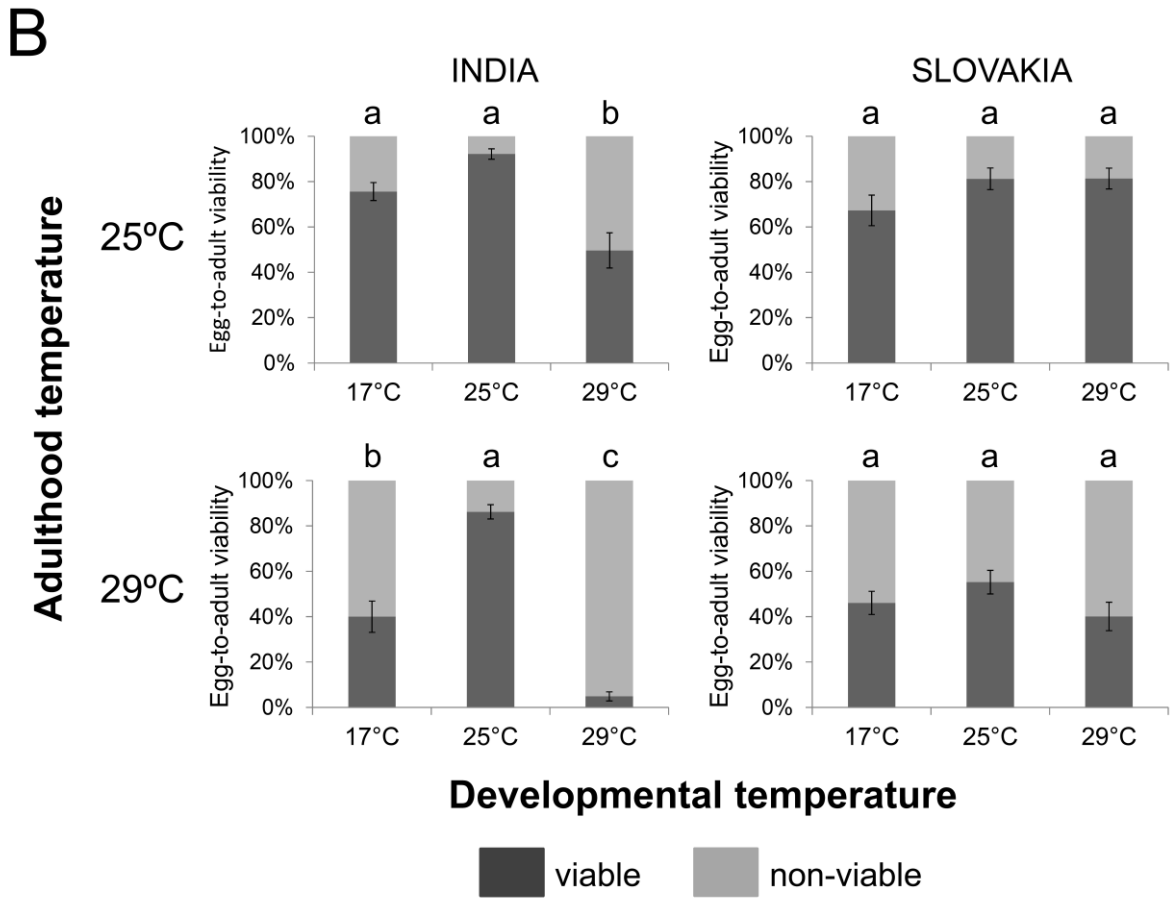

B
Adulthood temperature
25ºC
29ºC
INDIA
SLOVAKIA
a
b
c
Egg-to-adult viability
100%
80%
60%
40%
20%
0%
17°C
25°C
29°C
Developmental temperature
viable
non-viable

Figure S2. (A) Effect of developmental temperature of parents on the egg-to-adult viability at the day 20 at 17°C. (**B)** Effect of developmental temperature of parents on the egg-to-adult viability at the day 5 at 25°C and 29°C. Data for each population were compared by one-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$). Groups with the same letters are not significantly different from each other. Error bars represent s.e.m. For further statistical analyses see Table S3.

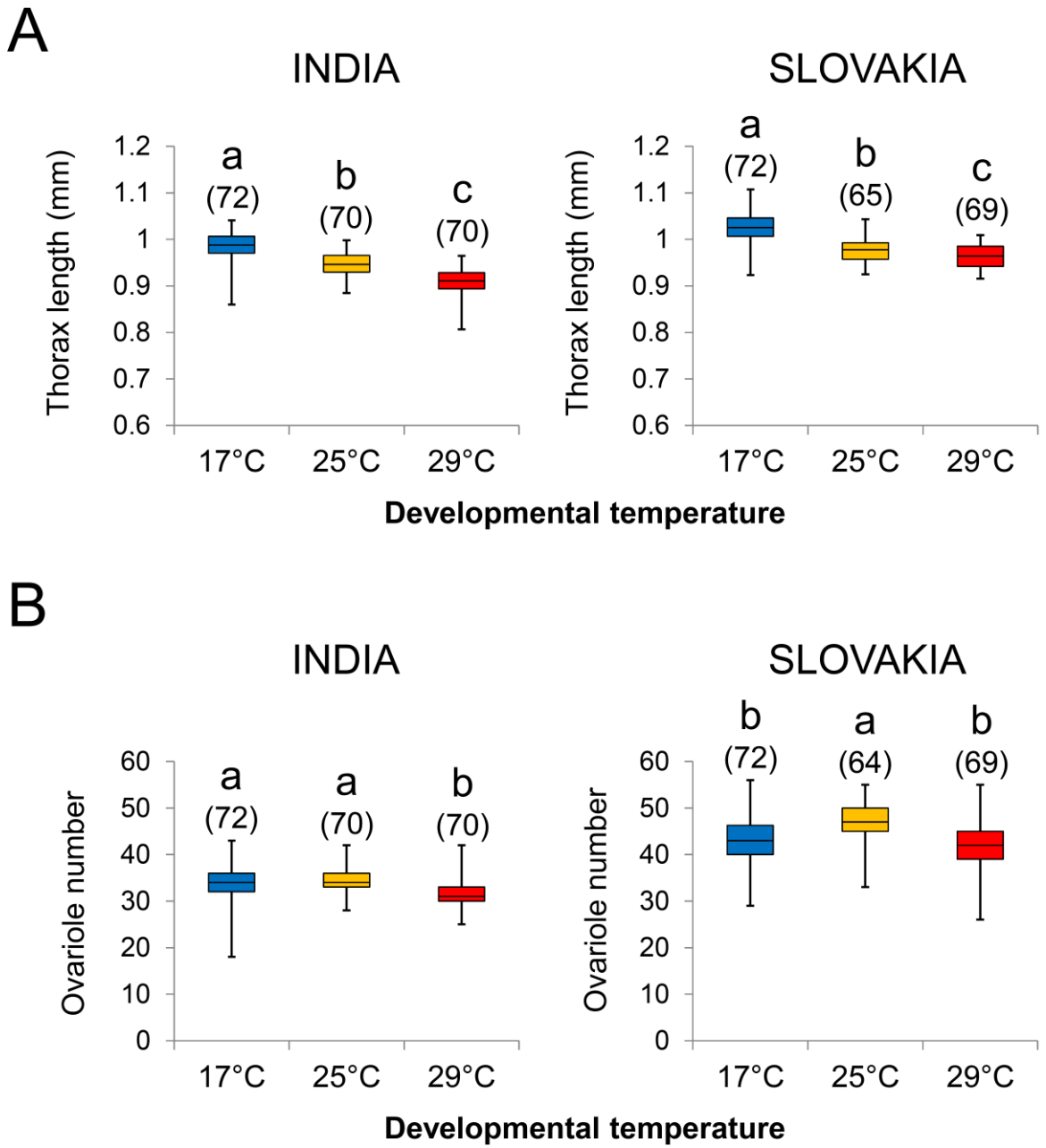


Figure S3. Effect of developmental temperature on thorax length (**A**) and ovariole number (**B**). Sample size is reported in parenthesis. Data for each trait and population were compared by one-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$). Groups with the same letters are not significantly different from each other. Box plots display minimum, first quartile, median, third quartile, and maximum value.

Table S1. Two-way analysis of variance (ANOVA) testing the effects of population and developmental temperature, and their interactions on early fecundity (the mean cumulative number of eggs laid per female during a given period) at three adulthood temperatures. *df* - degrees of freedom; SSQ - the sum of squares for each source of variation.

Adulthood temperature	Source of variation	*df*	SSQ	*F*-ratio	*P*-value
17°C (ten days)	Population	1	375185.1	120.58	< 0.0001
	Developmental temperature	2	286365.6	46.02	< 0.0001
	Population × Developmental temperature	2	103373.7	16.61	< 0.0001
	Error	135	420048.3	-	-
17°C (twenty days)	Population	1	1225051.9	72.80	< 0.0001
	Developmental temperature	2	1006284.7	29.90	< 0.0001
	Population × Developmental temperature	2	42492.2	1.26	0.286
	Error	135	2271642.8	-	-
25°C (ten days)	Population	1	1176651.3	51.43	< 0.0001
	Developmental temperature	2	364547.7	7.97	0.0005
	Population × Developmental temperature	2	15410.1	0.34	0.715
	Error	132	3020094.9	-	-
29°C (ten days)	Population	1	1703353.4	44.07	< 0.0001
	Developmental temperature	2	1371897.1	17.75	< 0.0001
	Population × Developmental temperature	2	106543.2	1.38	0.256
	Error	133	5140659.4	-	-

Table S2. Mean cumulative number of eggs (± s.e.m) laid by individual females during a given period, mean cumulative number of eggs (± SEM) per unit body size (thorax length^3), and mean cumulative number of eggs (± s.e.m) per ovariole at three adulthood temperatures. Data for each population, adulthood temperature and trait were compared by one-way ANOVA followed by Tukey's HSD test (α = 0.05). Groups with the same letters are not significantly different from each other.

Trait	Adulthood temperature	Population	Developmental temperature	Fecundity (10 days)	Tukey's HSD	Fecundity (20 days)	Tukey's HSD
Mean cumulative number of eggs per female	17°C	India	17°C	172.71 ± 7.95	A	399.46 ± 19.21	A
			25°C	155.71 ± 5.79	A	376.71 ± 15.40	A
			29°C	36.13 ± 10.62	B	190.87 ± 20.35	B
		Slovakia	17°C	200.92 ± 10.89	B	537.13 ± 28.74	A
			25°C	285.14 ± 18.61	A	587.82 ± 41.33	A
			29°C	188.17 ± 12.34	B	401.67 ± 29.57	B
	25°C	India	17°C	548.29 ± 21.23	B	-	-
			25°C	638.13 ± 18.68	A	-	-
			29°C	495.58 ± 22.28	B	-	-
		Slovakia	17°C	757.38 ± 47.07	A	-	-
			25°C	795.86 ± 44.04	A	-	-
			29°C	683.52 ± 21.91	A	-	-
	29°C	India	17°C	419.50 ± 32.77	B	-	-
			25°C	660.09 ± 27.50	A	-	-
			29°C	441.00 ± 34.37	B	-	-
		Slovakia	17°C	717.21 ± 47.18	A,B	-	-
			25°C	856.24 ± 57.96	A	-	-
			29°C	612.08 ± 41.00	B	-	-
Mean cumulative number of eggs per body size	17°C	India	17°C	177.74 ± 7.96	A	409.86 ± 18.12	A
			25°C	186.77 ± 7.98	A	450.82 ± 19.86	A
			29°C	48.94 ± 14.18	B	255.99 ± 27.70	B
		Slovakia	17°C	191.61 ± 11.32	B	511.09 ± 29.12	A,B
			25°C	295.17 ± 17.73	A	610.21 ± 41.08	A
			29°C	210.61 ± 12.67	B	450.84 ± 31.43	B
	25°C	India	17°C	571.58 ± 27.89	B	-	-
			25°C	762.89 ± 26.20	A	-	-
			29°C	655.57 ± 28.13	B	-	-
		Slovakia	17°C	688.18 ± 44.51	B	-	-
			25°C	857.48 ± 50.17	A	-	-
			29°C	748.95 ± 23.35	A,B	-	-
	29°C	India	17°C	449.47 ± 32.41	C	-	-
			25°C	761.86 ± 30.66	A	-	-
			29°C	595.44 ± 45.70	B	-	-
		Slovakia	17°C	660.58 ± 44.08	B	-	-
			25°C	938.77 ± 65.33	A	-	-
			29°C	691.74 ± 48.74	B	-	-
Mean cumulative number of eggs per ovariole	17°C	India	17°C	5.02 ± 0.21	A	11.64 ± 0.54	A
			25°C	4.57 ± 0.19	A	11.03 ± 0.45	A
			29°C	1.14 ± 0.33	B	6.07 ± 0.65	B
		Slovakia	17°C	4.83 ± 0.28	B	12.88 ± 0.70	A
			25°C	6.14 ± 0.41	A	12.64 ± 0.89	A
			29°C	4.45 ± 0.27	B	9.56 ± 0.68	B
	25°C	India	17°C	16.70 ± 0.70	B	-	-
			25°C	19.10 ± 0.53	A	-	-
			29°C	15.86 ± 0.67	B	-	-
		Slovakia	17°C	17.24 ± 1.05	A	-	-
			25°C	16.96 ± 0.87	A	-	-
			29°C	17.48 ± 0.79	A	-	-
	29°C	India	17°C	12.37 ± 0.81	B	-	-
			25°C	18.59 ± 0.67	A	-	-
			29°C	14.72 ± 1.14	B	-	-
		Slovakia	17°C	16.46 ± 1.04	A	-	-
			25°C	17.89 ± 1.25	A	-	-
			29°C	14.69 ± 1.06	A	-	-

Table S3. Two-way analyses of variance (ANOVA) testing the effects of population and developmental temperature, and their interactions on the egg-to-adult viability at a given day at three adulthood temperatures. *df* - degrees of freedom; SSQ - the sum of squares for each source of variation.

Adulthood temperature	Day	Source of variation	*df*	SSQ	*F*-ratio	*P*-value
17°C	10th	Population	1	0.80	17.57	< 0.0001
		Developmental temperature	2	3.32	36.54	< 0.0001
		Population × Developmental temperature	2	1.57	17.27	< 0.0001
		Error	123	5.58	-	-
	20th	Population	1	0.13	2.08	0.152
		Developmental temperature	2	1.17	9.22	0.0002
		Population × Developmental temperature	2	0.02	0.15	0.863
		Error	125	7.95	-	-
25°C	5th	Population	1	0.06	0.88	0.351
		Developmental temperature	2	1.08	7.95	0.0005
		Population × Developmental temperature	2	1.29	9.54	0.0001
		Error	132	8.94	-	-
	10th	Population	1	0.01	0.05	0.824
		Developmental temperature	2	0.46	2.19	0.116
		Population × Developmental temperature	2	0.26	1.23	0.297
		Error	122	12.71	-	-
29°C	5th	Population	1	0.04	0.69	0.407
		Developmental temperature	2	5.26	44.96	< 0.0001
		Population × Developmental temperature	2	2.47	21.13	< 0.0001
		Error	131	7.66	-	-
	10th	Population	1	0.01	0.10	0.752
		Developmental temperature	2	1.73	14.43	< 0.0001
		Population × Developmental temperature	2	0.80	6.72	0.002
		Error	122	7.30	-	-

Table S4. The analyses of early fecundity by ANCOVA with developmental temperature as the fixed factor and thorax length or ovariole number as the covariate. *df* - degrees of freedom; SSQ - the sum of squares for each source of variation.

Adulthood temperature	Population	Source of variation	*df*	SSQ	*F*-ratio	*P*-value	Source of variation	*df*	SSQ	*F*-ratio	*P*-value
17°C (ten days)	India	Developmental temperature	2	144236.1	43.78	< 0.0001	Developmental temperature	2	202903.0	63.67	< 0.0001
		Thorax length	1	84.8	0.05	0.821	Ovariole number	1	3701.8	2.32	0.132
		Error	67	110373.7	-	-	Error	67	106756.8	-	-
	Slovakia	Developmental temperature	2	129797.0	14.75	< 0.0001	Developmental temperature	2	80823.1	8.86	0.001
		Thorax length	1	19122.7	4.35	0.041	Ovariole number	1	8504.8	1.86	0.177
		Error	66	290467.0	-	-	Error	66	301084.9	-	-
17°C (twenty days)	India	Developmental temperature	2	285209.8	17.98	< 0.0001	Developmental temperature	2	480781.2	30.05	< 0.0001
		Thorax length	1	12767.6	1.61	0.209	Ovariole number	1	8209.9	1.03	0.315
		Error	67	531437.9	-	-	Error	67	535995.7	-	-
	Slovakia	Developmental temperature	2	276800.2	5.46	0.006	Developmental temperature	2	335898.7	6.57	0.003
		Thorax length	1	55693.6	2.20	0.143	Ovariole number	1	39619.3	1.55	0.218
		Error	66	1671743.6	-	-	Error	66	1687818.0	-	-
25°C (ten days)	India	Developmental temperature	2	230188.9	11.03	< 0.0001	Developmental temperature	2	179228.4	8.90	0.001
		Thorax length	1	335.6	0.03	0.858	Ovariole number	1	24793.1	2.46	0.121
		Error	67	699185.8	-	-	Error	67	674728.3	-	-
	Slovakia	Developmental temperature	2	161404.1	2.21	0.118	Developmental temperature	2	17287.9	0.26	0.774
		Thorax length	1	22225.4	0.61	0.438	Ovariole number	1	154777.6	4.62	0.036
		Error	63	2298348.0	-	-	Error	62	2078819.8	-	-
29°C (ten days)	India	Developmental temperature	2	847360.5	19.78	< 0.0001	Developmental temperature	2	505740.1	11.90	< 0.0001
		Thorax length	1	159193.2	7.43	0.008	Ovariole number	1	170993.6	8.05	0.006
		Error	66	1413944.6	-	-	Error	66	1402144.2	-	-
	Slovakia	Developmental temperature	2	686391.7	6.36	0.003	Developmental temperature	2	393642.1	3.62	0.032
		Thorax length	1	62008.8	1.15	0.288	Ovariole number	1	37056.2	0.68	0.412
		Error	65	3505512.8	-	-	Error	65	3530465.4	-	-

Table S5. Mean cumulative number of eggs (± s.e.m) laid by individual females during a given period at three adulthood temperatures adjusted either for thorax length or ovariole number. Adjusted values were compared by Tukey's HSD test (α = 0.05). Groups with the same letters are not significantly different from each other.

Adulthood temperature	Population	Developmental temperature	Adjusted for thorax length				Adjusted for ovariole number			
			Fecundity (10 days)	Tukey's HSD	Fecundity (20 days)	Tukey's HSD	Fecundity (10 days)	Tukey's HSD	Fecundity (20 days)	Tukey's HSD
17°C	India	17°C	171.17 ± 10.70	A	380.61 ± 23.48	A	170.57 ± 8.27	A	396.28 ± 18.53	A
		25°C	155.87 ± 8.32	A	378.70 ± 18.25	A	153.88 ± 8.24	A	373.99 ± 18.45	A
		29°C	37.56 ± 10.56	B	208.46 ± 23.17	B	40.26 ± 8.75	B	197.03 ± 19.62	B
	Slovakia	17°C	184.61 ± 15.64	B	509.29 ± 37.52	A,B	204.57 ± 14.04	B	545.01 ± 33.25	A
		25°C	286.28 ± 14.15	A	589.77 ± 33.96	A	277.58 ± 15.43	A	571.51 ± 36.53	A
		29°C	203.43 ± 15.39	B	427.71 ± 36.93	B	191.44 ± 13.99	B	408.73 ± 33.13	B
25°C	India	17°C	545.14 ± 27.29	B	-	-	544.61 ± 20.62	B	-	-
		25°C	638.47 ± 21.39	A	-	-	631.82 ± 21.31	A	-	-
		29°C	498.41 ± 26.15	B	-	-	505.31 ± 21.40	B	-	-
	Slovakia	17°C	784.88 ± 52.55	A	-	-	750.40 ± 37.52	A	-	-
		25°C	783.01 ± 43.93	A	-	-	757.73 ± 41.54	A	-	-
		29°C	665.57 ± 47.61	A	-	-	715.91 ± 42.71	A	-	-
29°C	India	17°C	372.47 ± 34.50	B	-	-	413.71 ± 29.82	B	-	-
		25°C	646.53 ± 30.92	A	-	-	626.27 ± 32.64	A	-	-
		29°C	503.63 ± 38.20	B	-	-	480.87 ± 33.48	B	-	-
	Slovakia	17°C	673.56 ± 62.48	A,B	-	-	722.26 ± 47.96	A,B	-	-
		25°C	874.81 ± 53.55	A	-	-	836.87 ± 56.00	A	-	-
		29°C	639.48 ± 53.85	B	-	-	623.98 ± 49.71	B	-	-