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Interspecific interactions alter the metabolic costs of climate warming

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Climate warming is expected to increase the energy demands of ectotherms by accelerating their metabolic rates exponentially. However, this prediction ignores environmental complexity such as species interactions. Here, to better understand the metabolic costs of climate change for ectotherms, we reared three *Drosophila* species in either single-species or two-species cultures at different temperatures and projected adult metabolic responses under an intermediate climate-warming scenario across the global range of *Drosophila*. We determined that developmental acclimation to warmer temperatures can reduce the energetic cost of climate warming from 39% to ~16% on average by reducing the thermal sensitivity of metabolic rates. However, interspecific interactions among larvae can erode this benefit of developmental thermal acclimation by increasing the activity of adults that develop at warmer temperatures. Thus, by ignoring species interactions we risk underestimating the metabolic costs of warming by 3–16% on average.

The current understanding of how ectotherms will respond to climate change is predominantly informed by research aimed at understanding the effect of temperature in isolation of other abiotic and biotic variables. For instance, rates of energy expenditure (metabolic rates) are known to accelerate approximately exponentially with acute increases in temperature due to the thermodynamics of the biochemical reactions that underlie metabolism[1]. The thermal sensitivity of ectotherm metabolic rates is often reduced following chronic exposure to warmer temperatures indicating that thermal acclimation can act to oppose the acute thermodynamic effect of temperature[2,3]. However, perfect thermal compensation that results in metabolic rate being insensitive to temperature change is rarely observed[2,3]. Thus, when cold- and warm-acclimated ectotherms are measured at the same temperature, warm-acclimated ectotherms are likely to have lower metabolic rates but when measured at their respective acclimation temperatures, warm-acclimated ectotherms often have higher metabolic rates. Consequently, the metabolic rates of ectotherms, and hence their energy demands, are expected to increase as the climate warms[2,4]. Increases in metabolic rate associated with climate warming may have important implications for ectotherm populations and communities because metabolic rate is related to growth rate, reproduction rate and longevity[5]. Together, these traits determine individual fitness and the intrinsic rate of growth[6] and carrying capacity[7–9] of populations, which in turn influence population persistence[10,11] and extinction risk[12]. However, evaluating the vulnerability of ectotherms to climate warming solely on the basis of their responses to temperature ignores the role of other processes, such as interspecific interactions, in shaping their energy balance.

Interspecific interactions are important forces in structuring communities and the direction of their effect on a species' fitness is often temperature sensitive[13–16]. Yet the current understanding of how interspecific interactions, such as interspecific competition, influence the metabolic rate of individuals is limited[17,18]. Density-mediated increases in intraspecific competition cause metabolic rates to decline[19] but individuals with higher metabolic rates can have greater fitness in environments where intraspecific and interspecific competition for resources is intense[18,20]. As such, competition (both within and among species) is an important biotic force in shaping individual metabolic rates but no study has yet investigated whether the presence of interspecific competitors alters the effect of temperature on the metabolic rate, mass and behaviour of ectotherms to determine how their overall energy demands might change in a warmer world. Furthermore, there is a need to understand whether interspecific interactions in the developmental environment can affect thermal acclimation responses that then carry over to affect adult metabolic rates and other fitness-related traits.

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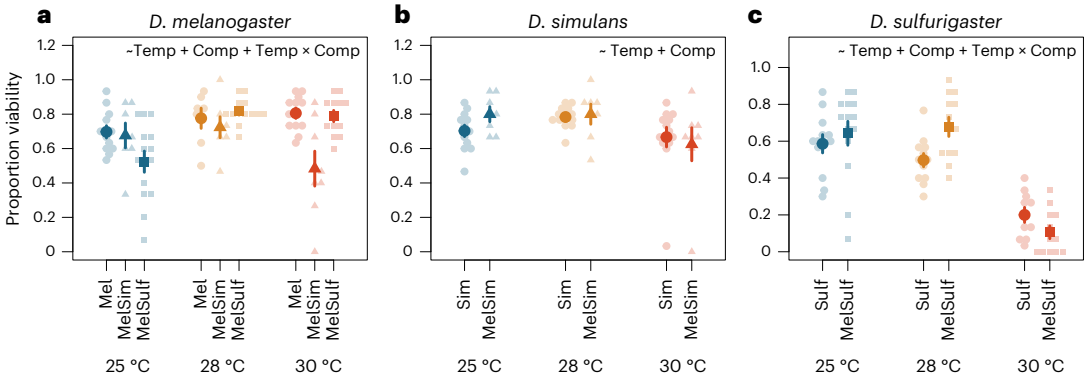

Fig. 1 | Effects of developmental conditions on egg-to-adult viability. a–c, The proportion egg-to-adult viability of Mel (**a**), Sim (**b**) and Sulf (**c**), in single-species or two-species cultures (competition treatment, Comp) of Mel and Sim (MelSim) or Mel and Sulf (MelSulf) at a mean temperature (Temp) of 25, 28 or 30 °C. Lighter points are replicate vials and darker points are group means (± s.e.). Each panel shows the combination of Temp and Comp in the minimum adequate model (Supplementary Tables 2–4).

Here, we examine how developing with interspecific competitors under simulated warming affects the metabolic rate, mass and behaviour of adult females of three *Drosophila* species (*D. melanogaster*, *D. simulans* and *D. sulfurigaster*) measured under common garden conditions. *Drosophila* larvae are an established model for studying competition and they compete both by reducing the amount of food available to others when food is limited (exploitation competition) and by harming each other through the excretion of waste products (interference competition)[21]. Thus, competitive interactions among *Drosophila* larvae are evident both when food is limited and when it is not. However, the waste products from larvae of one *Drosophila* species have also been shown to benefit the larvae of another species demonstrating that facilitation can also occur[21]. The three *Drosophila* species considered here vary in their geographic distribution—*D. melanogaster* and *D. simulans* are geographically widespread while *D. sulfurigaster* is restricted to the tropics—but all three species coexist where their distributions overlap (that is, they are attracted to the same fermenting fruit baits), which indicates that their larvae are likely to interact in nature[22,23].

To examine whether interspecific interactions in the larval environment alter the effect of developmental temperature on the metabolic rate, mass and behaviour of adults, we reared the three *Drosophila* species in single-species or two-species cultures (competition treatment) at a mean temperature of 25, 28 or 30 °C (temperature treatment), with a 5.5 °C range of daily fluctuations. For our two-species cultures, we paired *D. melanogaster* (Mel) with *D. simulans* (Sim) or *D. sulfurigaster* (Sulf). The mean temperatures of 25 and 28 °C and the 5.5 °C daily range broadly reflect the span of summer daily temperatures along the eastern coast of Australia where the three *Drosophila* species overlap in their distribution (Supplementary Table 1). We therefore expected that a mean temperature of 30 °C would be stressful for all three species and would represent a range of climate warming scenarios since it is both 5 and 2 °C warmer than our 25 and 28 °C treatments, respectively. To control for density effects, we established all cultures with 30 eggs (15 eggs per species in two-species cultures). Cultures were provided a limited supply of food that we assumed would promote competition among larvae (that is, intraspecific competition in single-species cultures and both intraspecific and interspecific competition in two-species cultures) but, as mentioned above, competition can occur even when food is not limited and facilitation can also occur. Thus, to assess whether interspecific interactions had a positive, negative or neutral effect on fitness-related traits, we counted the adults that emerged from each culture and calculated egg-to-adult viability as a proportion of the initial number of eggs of each species. If the viability of a species was lower or higher in two-species cultures than in its single-species cultures then we regarded this as evidence of interspecific competition or facilitation, respectively, but otherwise we attributed observed treatment effects to the presence of heterospecifics rather than interspecific competition or facilitation per se.

Following emergence, adult females were maintained under common garden conditions in single-species cultures at 25 °C for at least 3 days to examine the effects of developmental conditions independent of adult experience. We measured the metabolic rates of females (hereafter, absolute metabolic rates) as rates of carbon dioxide production at 25 °C using flow-through respirometry[24]. To disentangle the underlying mechanisms driving observed changes in absolute metabolic rates, we conducted simultaneous measures of mass and activity (a proxy for behaviour) and accounted for the variance associated with these traits in our statistical models to estimate: (1) the mass-independent metabolic rates of inactive animals to determine the effect of developmental conditions on the minimum energy costs of self-maintenance (hereafter, resting metabolic rate); and (2) the mass-independent metabolic rates of active animals to determine the effect of developmental conditions on the energy costs of performing voluntary routine activity (walking) in addition to self-maintenance (hereafter, routine metabolic rate) (Methods). To examine the direction and strength of the effect of interspecific interactions at each developmental temperature, we performed pair-wise comparisons by calculating competition indices for egg-to-adult viability and each adult trait where an index >0 indicates a higher trait value in the two-species culture and an index <0 indicates a higher trait value in the single-species culture (Methods).

Our data show that interspecific interactions in the larval environment alter the effects of developmental temperature on metabolic rates in all three species and that these effects are mediated by species-specific changes in their mass, behaviour and physiology.

Temperature effects in single-species cultures

In our single-species cultures, developmental temperature affected egg-to-adult viability and adult traits but these effects varied among the three *Drosophila* species. The widespread species, Mel and Sim, showed relatively high viability at all temperatures, whereas Sulf, which is restricted to the tropics and has the lowest heat tolerance of the three species[25,26], had reduced viability at 30 °C (Fig. 1 and Supplementary Tables 2–4). Consistent with previous research, adult Mel and Sim reared at 30 °C were, on average, 9% and 15% smaller (Fig. 2a,b), 31% and 50% less active (Fig. 2d,e), had 21% and 15% lower resting metabolic rates (Fig. 2g,h) and consequently had 23% lower routine metabolic rates (Fig. 2j,k) and 28% and 31% lower absolute metabolic rates (Fig. 2m,n), respectively, compared to those reared at 25 °C (Supplementary Tables 2 and 3)[24,27,28]. Since adults were maintained and

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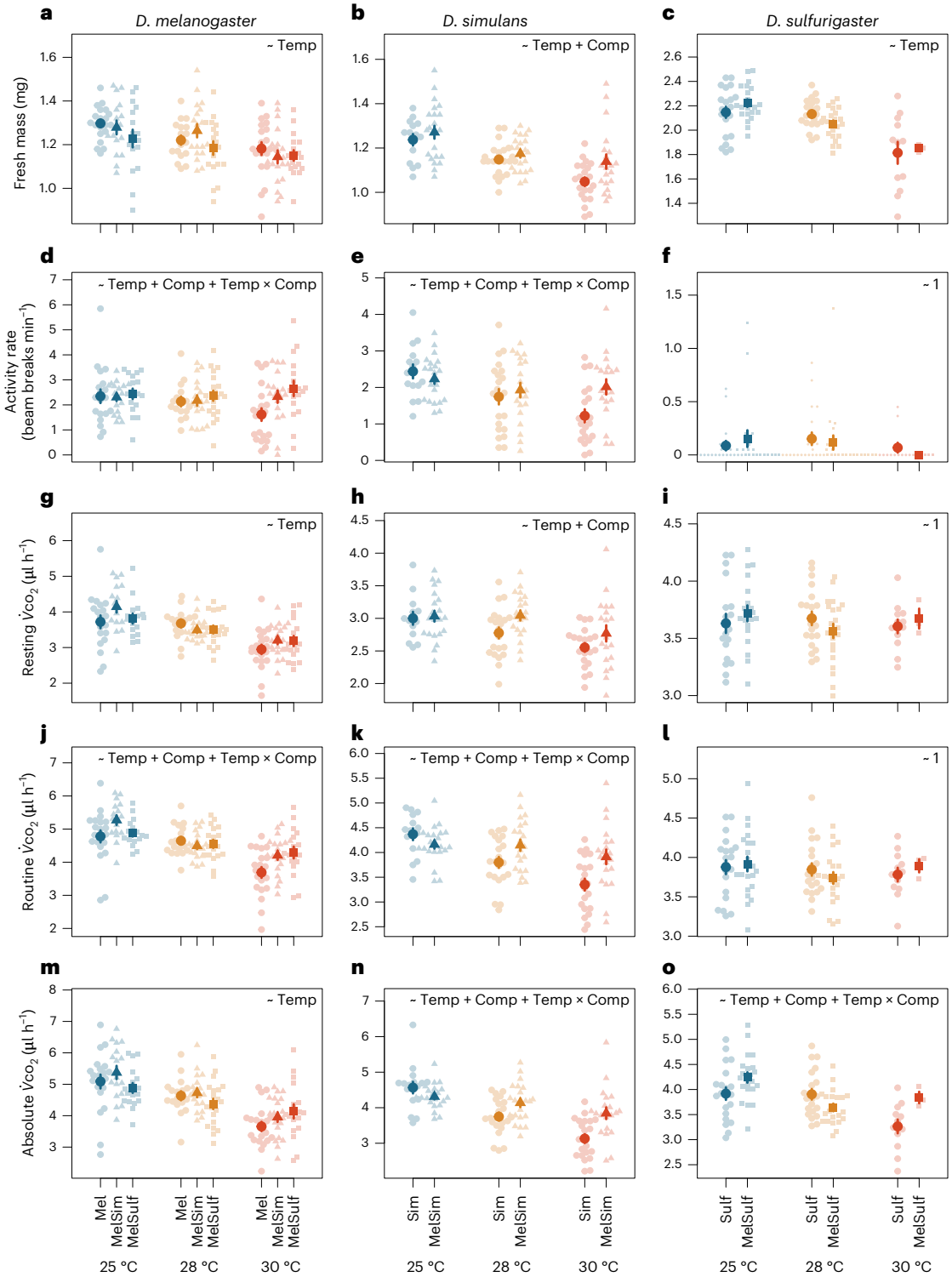

Fig. 2 | Effects of developmental conditions on adult traits. a–**o**, The mass (**a**–**c**), activity (**d**–**f**) and resting (**g**–**i**), routine (**j**–**l**) and absolute metabolic rates ($\dot{V}_{CO_2}$, µl h^{-1}) (**m**–**o**) of adult female Mel (**a**,**d**,**g**,**j**,**m**), Sim (**b**,**e**,**h**,**k**,**n**) and Sulf (**c**,**f**,**i**,**l**,**o**) after developing in single-species or two-species cultures (competition treatment, Comp) of Mel and Sim (MelSim) or Mel and Sulf (MelSulf) at a mean temperature (Temp) of 25, 28 or 30 °C. Light points are individual measurements (light points in **f** are smaller to show all data) and darker points are group means (± s.e.). Each panel shows the combination of Temp and Comp in the minimum adequate model (Supplementary Tables 2–4). Data points for resting and routine $\dot{V}_{CO_2}$ are the measured $\dot{V}_{CO_2}$ values standardized to the species-specific mean mass (Mel, 1.21 mg; Sim, 1.17 mg; and Sulf, 2.09 mg) and for resting $\dot{V}_{CO_2}$, data points are also standardized to zero activity using the parameter estimates for mass and activity rate in the minimum adequate models. Data points for resting, routine and absolute $\dot{V}_{CO_2}$ are also adjusted for the fixed effect of work station and adjusted to the mean random intercept for measurement block and channel if these were included in the minimum adequate model.

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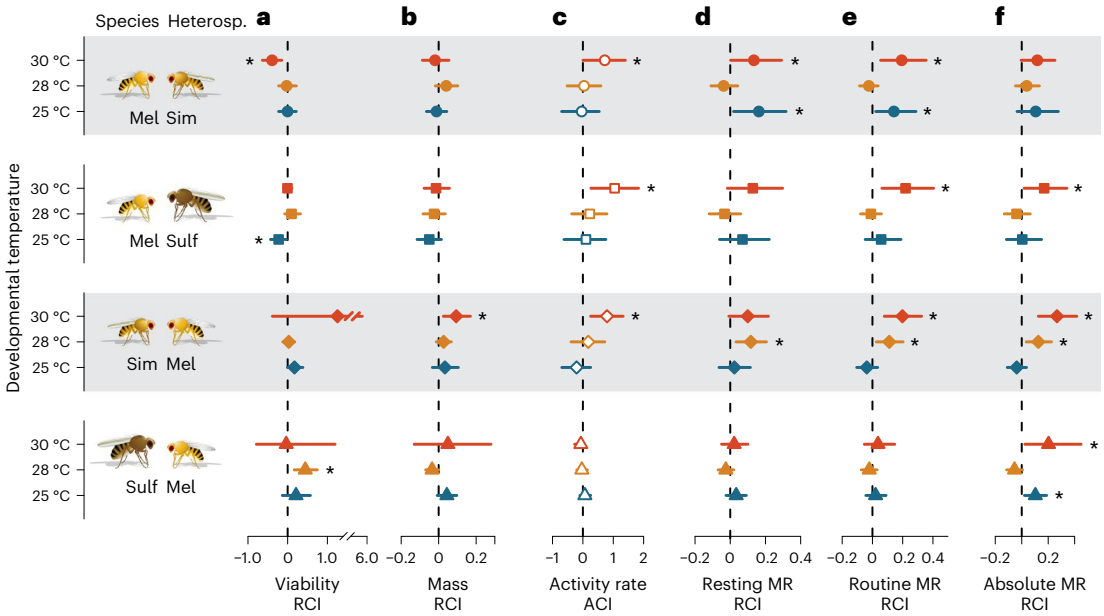

Fig. 3 | Competition indices. a–f, RCI and ACI for Mel, Sim and Sulf showing the direction and strength of the effect of larval interspecific interactions on egg-to-adult viability (**a**), adult mass (**b**), activity (**c**) and resting (**d**), routine (**e**) and absolute metabolic rate (MR) (**f**) at each mean developmental temperature. Indices were calculated for each species (left) to compare trait values between its single-species culture and its two-species culture with a heterospecific (Heterosp.; right). Data shown are means and 95% confidence intervals calculated by 10,000 bootstraps with replacement. Stars denote where confidence intervals do not cross zero.

measured at 25 °C, these decreases are indicative of developmental acclimation responses to warming with the decreases in resting metabolic rate indicating that thermal acclimation probably acts to oppose the acute thermodynamic effect of temperature on physiological rates. Adult Sulf that developed at 30 °C were also 15% smaller (Fig. 2c) and consequently had 17% lower absolute metabolic rates (Fig. 2o) on average compared to those reared at 25 °C but the adults of this species were relatively inactive irrespective of developmental conditions (Fig. 2f) (Supplementary Table 4). Consequently, the resting and routine metabolic rates of Sulf were effectively the same and, unlike in the other species, these metabolic rates were not significantly affected by developmental temperature (Fig. 2i,l and Supplementary Table 4), which is consistent with the hypothesis that tropical ectotherms have little capacity for thermal acclimation because the tropics are more thermally stable[29].

Temperature effects on interspecific interactions

Larval *Drosophila* interact through the consumption of shared food resources (exploitation competition) as well as through the excretion of waste products that can either be harmful (interference competition) or beneficial (facilitation)[21]. In agreement with previous studies in *Drosophila*, we found that the nature of the interaction between species varied with temperature[13,15]. We found evidence of interspecific competition between Mel and Sim at 30 °C and between Mel and Sulf at 25 °C because the egg-to-adult viability of Mel was reduced in both cases (Figs. 1a and 3a and Supplementary Table 2). Complementary to this, we found that the adult mass of Sim and Sulf was higher after developing with Mel at 30 and 25 °C, respectively, although the increase in mass was not statistically significant for Sulf (Figs. 2b,c and 3b and Supplementary Tables 3 and 4).

In contrast, the presence of Mel increased the viability of Sulf at 28 °C, indicating facilitation (Figs. 1c and 3a and Supplementary Table 4). Mel may have benefitted Sulf by excreting useful metabolites[30] or through other effects on the food. Other *Drosophila* species benefit from the presence of Mel in the larval environment because Mel slows food drying[31] and can inhibit the growth of mould[32], both of which may have been more prone to occur at warmer temperatures. At 30 °C, however, Sulf suffered high mortality irrespective of whether it was reared with or without Mel and thus it is unclear if interspecific competition or facilitation occurred (Figs. 1c and 3a and Supplementary Table 4).

Although we did not find evidence of interspecific competition within our two-species cultures at all temperatures, the absence of clear signatures of competition on viability or adult mass does not mean that larvae were not competing. It is possible that the presence of interspecific competitors had negative effects on fitness traits not measured in the present study, such as development rate and adult reproductive success. However, irrespective of whether interspecific competition was occurring or not, it is clear that interspecific interactions in the larval environment can have temperature-specific consequences for metabolic rates in adults (discussed below).

Temperature effects in two-species cultures

For all species, interspecific interactions in the larval environment did not have a particularly strong influence on the effect of developmental temperature on adult mass or resting metabolic rate given the lack of a statistically significant interaction between temperature and competition treatment (Fig. 2a–c,g–i and Supplementary Tables 2–4). However, our competition indices suggested that, at some temperatures, interactions between Mel and Sim increased the adult mass of Sim (by 9% on average at 30°C, Fig. 3b) and increased the resting metabolic rate of Mel (by 16% on average at 25 °C and by 13% on average at 30 °C; Fig. 3d) and Sim (by 12% on average at 28 °C; Fig. 3d).

Unlike resting metabolic rate, interspecific interactions significantly altered the effect of developmental temperature on the routine metabolic rate of adult Mel and Sim and the absolute metabolic rate of Sulf (Fig. 2j,k,o and Supplementary Tables 2 and 4). In two-species cultures, adult Mel and Sim reared at 30 °C had 12–20% and 6% lower routine metabolic rates on average, respectively, compared to those reared at 25 °C, whereas in single-species cultures, their routine metabolic

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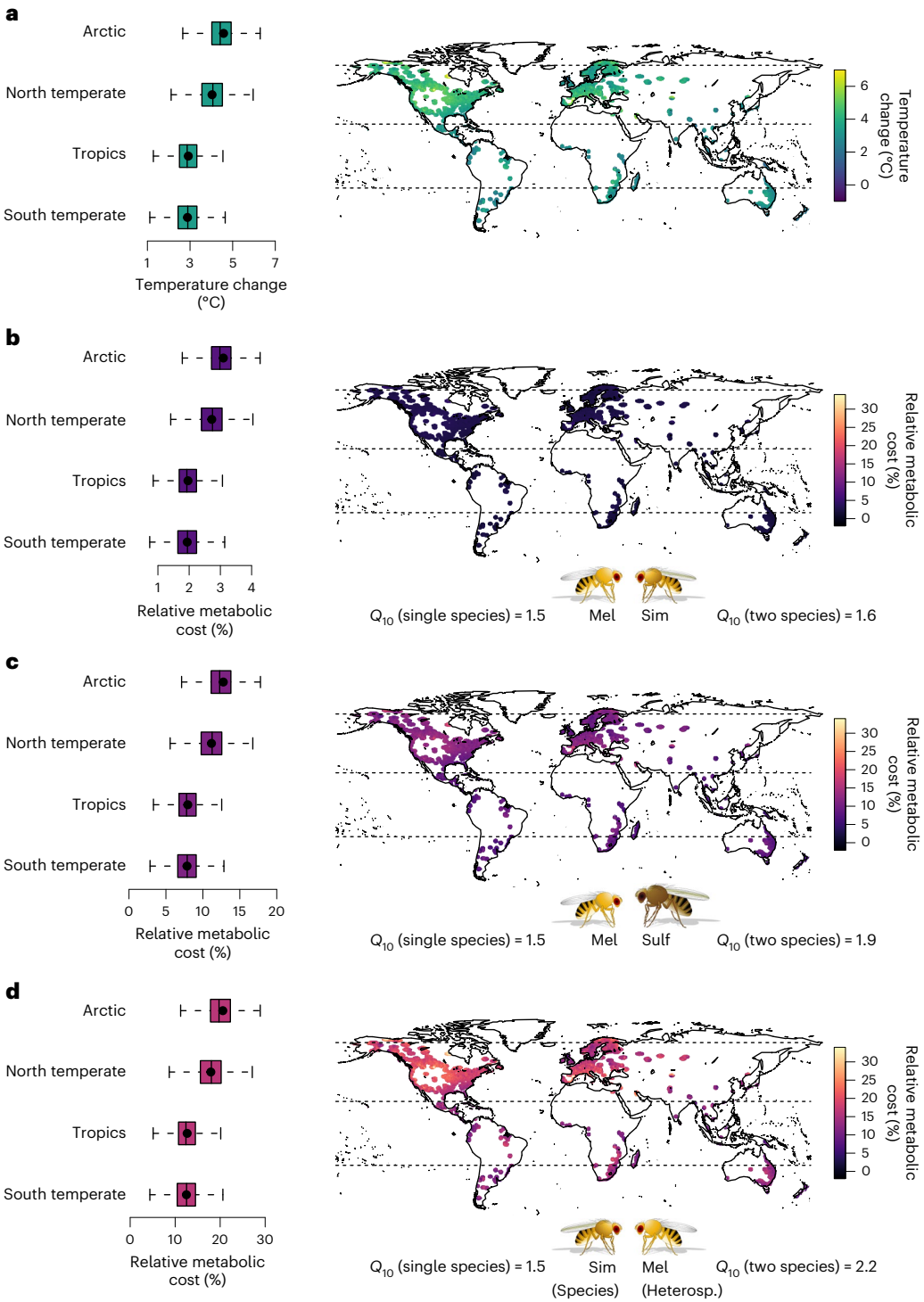

Fig. 4 | Relative metabolic cost of interspecific interactions under climate warming. Spatially explicit predictions of the relative metabolic cost (%) of interspecific interactions at the end of the century under an intermediate climate-warming scenario (SSP 2-4.5) across the global range of *Drosophila* (n = 1,944,000). **a**, Changes in temperature at each location were calculated between recent (1970–2000) and projected future (2081–2100) climates using the mean temperature of the warmest quarter at a 10 arcmin resolution. The warmest quarter is assumed to be when *Drosophila* are most active. Future temperatures were extracted from climate projections (CMIP6) based on eight global climate models (BCC-CSM2-MR, CanESM5, CNRM-CM6-1, CNRM-ESM2-1, IPSL-CM6A-LR, MIROC-ES2L, MIROC6 and MRI-ESM2-0) under the SSP 2-4.5 scenario. **b**–**d**, Predictions are based on the thermal sensitivities of the routine metabolic rate (described by Q_{10} values) of Mel (**b**,**c**) and Sim (**d**) (left) following developmental thermal acclimation in single-species or two-species cultures with a heterospecific (Heterosp.; right). For Mel, the heterospecific was either Sim or Sulf. For Sim, the heterospecific was Mel. Metabolic costs of interspecific interactions under climate warming are expressed as the percentage difference between predicted metabolic rates with and without interspecific interactions relative to predicted metabolic rates without interspecific interactions (equation (9)). Predictions are constrained to within 200 km of occurrence localities. Data are summarized for each region with boxes showing the interquartile range (IQR), lines within boxes showing the median, whiskers showing the 1.5×IQR range and points are the mean (Arctic, n = 304,560; north temperate, n = 559,440; tropics, n = 604,800; south temperate, n = 475,200). Outliers are excluded from box plots for visual clarity. Coastline data from mapdata v.2.3.0 (ref. [36]).

rates declined by more (23%) (Fig. 2j,k and Supplementary Tables 2 and 3). As mentioned previously, because adults were measured at 25 °C, these decreases in routine metabolic rate are indicative of developmental acclimation responses to warming but these acclimation responses are less pronounced for Mel and Sim reared in two-species cultures. This interactive effect of temperature and species interactions on the routine metabolic rate of Mel and Sim arose mostly from effects observed at 30 °C. Adult Mel and Sim reared at 30 °C in two-species cultures were 44–65% and 65% more active on average, respectively (Figs. 2d,e and 3c), and consequently had 14–16% and 17% higher routine metabolic rates on average (Figs. 2j,k and 3e), respectively, compared to those reared at 30 °C in single-species cultures. Interactions with Mel caused the absolute metabolic rate of Sulf to increase by 8% and 18% on average at 25 and 30 °C, respectively (Figs. 2o and 3f). Unlike Mel and Sim, these changes in the absolute metabolic rate of Sulf were not driven by changes in activity, rather they were probably due to subtle cumulative effects of developmental conditions on the adult mass (Figs. 2c and 3b) and resting metabolic rate (Figs. 2i and 3d) of Sulf.

While the role of interspecific interactions in shaping metabolic responses to warming has not been previously investigated, our findings are consistent with other studies on Mel that demonstrate that the thermal and resource conditions in the developmental environment can affect adult behaviour and metabolic rates (carryover effects)[24,33]. Most notably we found that developing with heterospecifics at 30 °C increases the adult activity of Mel and Sim and consequently this causes their routine metabolic rate to increase but whether exploitative or interference competition or some other mechanism associated with interspecific interactions is responsible for this change in behaviour is unknown. High larval density is known to increase the feeding behaviour and mating-induced aggression of adult females in Mel and this is thought to arise as a consequence of increased intraspecific competition for limited food that primes individuals for increased adult competition[33]. Reductions in food quantity or quality arising from the presence of interspecific competitors or the increased presence of decaying carcasses in two-species cultures at 30 °C may have similarly caused the changes in adult female activity of Mel and Sim in the present study. Alternatively, the changes in adult activity we observed may have been due to the presence of heterospecific pheromones that signal information about the social environment and elicit behavioural responses in adults[34]. However, further research is needed to establish the mechanistic basis of observed changes in adult behaviour caused by the presence of larval heterospecifics.

Metabolic costs of climate warming

Our experimental results highlight that examining the responses of ectotherms to warmer temperatures under more ecologically realistic conditions where species must interact is likely to alter and improve our understanding of how climate warming may impact the energy demands of natural populations. To examine how effects of the magnitude we observed will affect predictions of the metabolic costs of climate warming across the range of climates that *Drosophila* inhabit, we modelled future metabolic rates across the global range of *Drosophila* with and without interspecific interactions under an intermediate climate-warming scenario (shared socio-economic pathway, SSP 2-4.5). We parameterized the thermal sensitivity of metabolic rate as the factorial change in routine metabolic rate relative to a 10 °C change in temperature (Q_{10}). We used measures of routine metabolic rate because interspecific interactions most clearly affected the temperature-dependence of metabolic rates via changes in routine activity and had minimal effects on mass and resting metabolic rate. Following the thermal sensitivity model of ref. [2] (Extended Data Fig. 1), we calculated Q_{10} values on the basis of the thermal acclimation responses observed in our experimental treatments (acclimation Q_{10} values ranged from 0.6 to 1) multiplied by an acute Q_{10} value of 2.5 (Supplementary Table 5), which we derived from the acute thermal responses observed in Mel reared and measured at temperatures ranging from 16 to 30 °C (ref. [27]) (Methods). Since routine metabolic rate in our study is a mass-independent measure of metabolic rate, we expressed metabolic rates in units of mW $g^{-0.75}$ in accordance with metabolic theory[1] and data[35].

Our models show that ignoring interspecific interactions can significantly underestimate the future energy demands of ectotherms under climate warming. First, models that ignore interspecific interactions support the conclusion that physiological acclimation can increase the resilience of ectotherms to climate change by reducing the thermal sensitivity of their metabolic rate[2]. We predict that for those *Drosophila* species with limited capacity for acclimation, such as Sulf, climate warming will increase their metabolic rates by 39% on average (Supplementary Table 6). For those species that can acclimate, such as Mel and Sim, the metabolic costs of warming will be substantially less and limited to ~16% on average (Supplementary Table 6). However, because interspecific interactions can erode the energetic benefits of acclimation by increasing the activity of adults that develop at warmer temperatures, we predict that interspecific interactions will increase the metabolic costs of climate warming by an additional 3–16% on average (Fig. 4 and Supplementary Table 6). Due to the exponential nature of the thermal sensitivity of metabolic rate[4], we find that the effect of interspecific interactions on the absolute metabolic costs of warming is greatest in the tropics despite the magnitude of forecasted warming being smaller in this region (Extended Data Fig. 2). By incorporating the effect of interspecific interactions on behaviour in our model projections, we show that there is a risk of systematically underestimating the energy demands of ectotherms in a warmer world if predictions continue to be based solely on physiological responses to temperature. However, interspecific interactions did not consistently affect the thermal sensitivity of our measures of metabolic rate and varied among the three *Drosophila* species considered here. We therefore encourage further research to improve the understanding of the combined effects of temperature and interspecific interactions on the physiology, behaviour and morphology of ectotherms adapted to a range of climates.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41558-023-01607-6.

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Methods

Fly stocks

Field-inseminated females of Mel and Sim were collected from Melbourne, Australia (37.8136° S, 144.9631° E) and Sulf were collected from Cairns, Australia (16.8692° S, 145.6749° E) between March and May 2017. For each species, 20 field-inseminated females were isolated in separate culture vials and the resulting progeny were used to establish a second generation (an isofemale line). From the second generation, 10 virgin females and males from each of the 20 isofemale lines were placed into a bottle to establish a mass-bred population. Mass-bred populations of each species were maintained in the laboratory in three bottles containing a yeast-potato-dextrose-agar medium at 25 °C under 12:12 h light:dark cycle at an approximate census size of 750–1,000. On initiation of the experiment, Mel had been maintained in the laboratory for 11 generations, while Sim and Sulf had been maintained for 8 generations.

Experimental treatments

Experimental flies were obtained by placing flies from our fly stocks into egg-laying chambers and allowing them to oviposit for 12 h on the same yeast-potato-dextrose-agar medium as our fly stocks but with added blue food dye and double the agar. The eggs laid in these chambers were used to establish single-species cultures of each species and two-species cultures of Mel and Sim or Mel and Sulf (competition treatment). We did not consider a two-species culture of Sim and Sulf because Mel was the focal species for our study. Single-species cultures were established with 30 eggs while two-species cultures were established with 15 eggs of each species. Eggs were placed into vials containing 1.5 ml of the yeast-potato-dextrose-agar medium because pilot experiments indicated that the larvae from 30 eggs deplete ~1.5 ml of food from hatching to adult emergence. Replicate vials (n = 7–14) of each culture were maintained at one of three fluctuating temperature regimes with a mean temperature of 25 °C (range 22.5–28 °C), 28 °C (range 25.5–31 °C) or 30 °C (range 27.5–33 °C) (temperature treatment) (Supplementary Table 5).

Egg-to-adult viability

The adults that successfully eclosed within each vial were collected within 12 h of emergence and counted and sexed under CO_2 anaesthesia. For each vial, we calculated the egg-to-adult viability of a species as the proportion of the initial number of eggs of the species in a culture (30 eggs in single-species cultures or 15 eggs in two-species cultures) that successfully emerged as adults.

Adult maintenance and trait measurements

The females collected from at least five replicate vials were maintained at 25 °C in vials with 3 ml of yeast-potato-dextrose-agar medium in groups of 10 individuals without access to males for at least 3 days before metabolic rate measurements. However, because of high mortality of Sulf at 30 °C, there were 12 individuals maintained in one vial from the single-species culture and only 3 individuals from the two-species culture maintained in one vial. By maintaining females under these common garden conditions, we were able to establish the effects of developmental conditions independent of adult experience. Only females were retained for measurement due to logistical constraints and because the effect of developmental temperature on the acute thermal sensitivity of adult metabolic rates has been well characterized for female *D. melanogaster*[27], which facilitated the modelling of the metabolic costs of interspecific interactions under climate warming (discussed below).

The rates of CO_2 production ($\dot{V}_{CO_2}$, µl h^{-1}) of individual adult virgin female flies at 25 °C were measured as a proxy for metabolic rate using a 14-channel flow-through respirometry (indirect calorimetry) system described by ref. [24] (Supplementary Note). The activity of individual flies was measured simultaneously using *Drosophila* activity monitors (DAM) that counted the number of times a fly broke an infrared beam when it walked past the midpoint of the respirometry chamber, which was a plastic tube with a 5 mm diameter and 45 mm of tube length available for voluntary walking locomotion. The $\dot{V}_{CO_2}$ and activity of 14 flies was measured in one measurement block with eight respirometry chambers inserted into one DAM and six respirometry chambers inserted into a second DAM. Each DAM was placed inside one of two temperature-controlled cabinets that maintained temperature to 25 ± 1 °C and kept flies in the dark. The $\dot{V}_{CO_2}$ and activity of each fly was measured for 25 min following a 40 min settling period without food and flies were weighed immediately following metabolic rate measurements. Measurements were conducted in a randomized order over a period of 2 days across 28 measurement blocks (14 blocks per day) with all treatments measured on each day. Flies were 4–6 and 5–7 days of age on the first and second day of measurement, respectively. In total, we measured the $\dot{V}_{CO_2}$, activity and mass of 382, 368 and 376 flies, respectively, giving us a sample size of 13–21 for each adult trait, except in the case of Sulf that developed at 30 °C where we were only able to measure 12 and 3 individuals from the singles-species and two-species cultures, respectively, due to high mortality at this temperature.

For each 25 min $\dot{V}_{CO_2}$ and activity recording, the first 5 min of data were discarded to allow flies to resettle in the chambers following connection of chambers to the respirometry system. The $\dot{V}_{CO_2}$ averaged over the final 20 min was taken as the measure of absolute and routine metabolic rate for each fly and activity recorded over this same 20 min period was taken as the measure of activity for each fly. The lowest $\dot{V}_{CO_2}$ averaged over 10 min and the activity recorded over this same 10 min period were used to estimate resting metabolic rate. This protocol for measuring activity in flies is sufficient to characterize the total activity of flies over 24–72 h because for Mel and Sim there is a significant positive correlation between short (10 and 20 min) and long (24–72 h) activity measurements (Supplementary Fig. 1), whereas Sulf is mostly inactive over 72 h with activity observed in only 0.05–3.4% of minute-long measurements in 29 individuals (Supplementary Table 8).

Statistical analyses

All data were analysed using R v.4.2.0 (ref. [37]). The interactive effects of temperature and competition treatment on egg-to-adult viability and adult traits were analysed separately for each species. Generalized linear models were fit to proportion viability data (proportion viable and dead in each vial) using the glmmTMB function of the glmmTMB package v.1.1.4 (ref. [38]) with a betabinomial error distribution and a logit link function and, for Mel and Sim, zero inflation that varied as a function of the fixed predictors in the full model. Linear mixed models were fit to mass and metabolic rate data with maximum likelihood using the lmer function of the lme4 package v.1.1-30 (ref. [39]). Generalized linear mixed models were fit to count data for activity with maximum likelihood using the glmmTMB function of the glmmTMB package v.1.1.4 (ref. [38]) with the natural log of measurement duration as an offset, a negative binominal (linear parameterization) family distribution and, for Mel, zero inflation that varied as a function of the fixed predictors in the full model.

All full models included an interaction term between the fixed categorical factors of temperature (25, 28 or 30 °C) and competition treatment. For Mel there were three levels within the fixed factor of competition treatment: (1) the control single-species culture; (2) the two-species culture with Sim; and (3) the two-species culture with Sulf. For Sim and Sulf, there were two levels within the fixed factor of competition treatment: (1) the control single-species culture and (2) the two-species culture with Mel. Models used to analyse adult trait data included work station as a fixed categorical factor with two levels (station 1 and 2; each station consisted of a temperature-controlled cabinet and DAM) and random intercepts for the categorical factors of measurement channel (1–14) and measurement block (1–28). Metabolic

rate data were analysed either with mass and activity, mass only or without mass and activity as continuous covariates to determine treatment effects on resting, routine and absolute metabolic rate, respectively.

Full generalized linear mixed models were reduced using stepwise backwards elimination of random-effect terms followed by stepwise backward elimination of fixed-effect terms based on Akaike's information criterion using the AICtab function of the bbmle package v.1.0.25 (ref. [40]). Full linear mixed models were reduced using backward elimination of random-effect terms ($\alpha = 0.1$) followed by backward elimination of fixed-effect terms ($\alpha = 0.05$) with denominator degrees of freedom calculated using Satterthwaite's method using the step function of the lmerTest package v.3.1-3 (ref. [41]). The significance of fixed effects in minimum adequate models was tested using Type-III Wald χ^2 tests for generalized linear models, linear mixed models and generalized linear mixed models and Type-III F-tests for linear models using the Anova function of the car package v.3.1-0 (ref. [42]). The tests of significance for parameter estimates in general linear models were performed using z-tests. In linear models they were performed with t-tests and in linear mixed models they were performed with t-tests using the Satterthwaite's method for degrees of freedom in the lmerTest package v.3.1-3 (ref. [41]). Model assumptions were tested using the DHARMa package v.0.4.5 (ref. [43]).

To determine the resting metabolic rate of flies, the parameter estimates for mass and activity in the minimum adequate models were used to adjust measures of metabolic rate to the mean adult mass of each species (Mel, 1.21 mg; Sim, 1.17 mg; and Sulf, 2.09 mg) and to zero activity (mass-independent values for inactive animals). To determine the routine metabolic rate of flies, the parameter estimate for mass in the minimum adequate models was used to adjust measures of metabolic rate to the mean adult mass of each species (Mel, 1.22 mg; Sim, 1.17 mg; and Sulf, 2.09 mg) (mass-independent values of active animals). Resting, routine and absolute metabolic rate data were also adjusted for the fixed effect of work station and adjusted to the mean random intercept for measurement block and channel if these were included in the minimum adequate model.

We performed pair-wise comparisons between our single- and two-species cultures at each temperature by calculating a relative competition index (RCI) for fresh mass and resting, routine and absolute metabolic rate using equation (1) and an absolute competition index (ACI) for activity using equation (2):

$$\mathrm{RCI} = \frac{X_{\mathrm{Comp}} - X_{\mathrm{Control}}}{X_{\mathrm{Control}}} \quad (1)$$

$$\mathrm{ACI} = X_{\mathrm{Comp}} - X_{\mathrm{Control}} \quad (2)$$

where X_{Comp} and X_{Control} are randomly sampled trait values from the two-species competition treatment and the single-species control treatment, respectively. An RCI could not be calculated for activity because activity rate data included zero values, which would yield undefined values when the selected value for X_{Control} (the denominator) was zero. Competition indices >0 indicate a higher trait value in the two-species competition treatment and an index <0 indicates a higher trait value in the single-species control treatment. Means and 95% confidence intervals for the competition indices were calculated by 10,000 bootstraps using the sample function in R with replacement.

Modelling the metabolic costs of climate warming

To model the metabolic costs of interspecific interactions under climate warming we followed the thermal sensitivity model of ref. [2] and parameterized the thermal sensitivity of metabolic rate as the factorial change in routine metabolic rate relative to a 10 °C change in temperature (Q_{10}) (Extended Data Fig. 1). To do this, we first explored how developmental temperatures ranging from 16 to 30 °C affected the acute thermal sensitivity of the routine metabolic rate of adult female Mel measured at 16 and 30 °C. The metabolic rate data we used to explore this were from a previous study by ref. [27], which was conducted using similar collection, population maintenance and respirometry protocols as that used in the present study. Briefly, 30 field-inseminated female Mel were collected from Melbourne, Australia (37.8136° S, 144.9631° E) in 2014 and were used to establish a mass-bred population that was maintained in the laboratory at 25 °C for 7–9 generations. The $\dot{V}_{CO_2}$ of adult female Mel that were 5–8 days of age were measured at 16, 25 or 30 °C after developing in vials on a yeast-potato-dextrose-agar medium at one of six developmental temperatures (16, 18, 22, 25, 28 and 30 °C). Metabolic rate data were $\log_{10}$ transformed and analysed in a linear model using the lm function with an interaction term between the fixed factors of developmental temperature, measurement temperature and the continuous covariate of $\log_{10}$-transformed mass. The model was then reduced using backward elimination of fixed-effect terms ($\alpha = 0.05$) with denominator degrees of freedom calculated using Satterthwaite's method using the step function of the lmerTest package v.3.1-3 (ref. [41]). The minimum adequate model that explained the observed variation in metabolic rate included $\log_{10}$ mass and a significant interaction between developmental and measurement temperature. The parameter estimate for $\log_{10}$ mass (parameter estimate ± s.e., s.e. 0.59 ± 0.11, $t_{964} = 5.21$, $P < 0.001$) was used to adjust measures of metabolic rate to the mean adult mass (1.19 mg) to calculate their routine metabolic rate. We calculated the acute Q_{10} value for each developmental temperature to describe the change in mean routine metabolic rate between the measurement temperatures of 16 and 30 °C. We found that the acute Q_{10} value varied across a narrow range from 2.3 to 2.7 among the six developmental temperatures. We therefore assumed that the acute Q_{10} value of routine metabolic rate was (1) independent of developmental conditions, (2) the same for all *Drosophila* species used in the present study and (3) equal to the mean of these acute Q_{10} values (2.5) (Extended Data Fig. 1). We consider these assumptions regarding the acute Q_{10} value to be reasonable given that the acute Q_{10} value has been shown not to vary among 65 *Drosophila* species originating from climates with mean annual temperatures ranging from 4 to 26 °C (ref. [44]).

To account for the developmental thermal acclimation responses observed in the present study where adult flies were measured at 25 °C, we calculated acclimation Q_{10} values for each species to describe the change in mean routine metabolic rate of adult flies reared at 25 and 30 °C for each control and competition treatment (Extended Data Fig. 1 and Supplementary Table 5). The acclimation Q_{10} values for Mel and Sim were <1 indicating that when measured at the same temperature, acclimation to warmer temperatures reduces their metabolic rate. The acclimation Q_{10} values of those reared in two-species cultures were greater than those reared in single-species cultures indicating that interspecific interactions weakened the thermal acclimation response of metabolic rate (Supplementary Table 5). Because the routine metabolic rate of Sulf was unaffected by developmental conditions, its acclimation Q_{10} values for the single-species and two-species cultures were similar and close to 1 (Supplementary Table 5). Following the thermal sensitivity model of ref. [2], we multiplied these acclimation Q_{10} values by the acute Q_{10} value of 2.5 to estimate post-acclimation Q_{10} values, which describe the change in metabolic rate between cold- and warm-acclimated animals measured at their respective acclimation temperatures (Extended Data Fig. 1 and Supplementary Table 5). In other words, the post-acclimation Q_{10} value describes the sensitivity of metabolic rate to changes in temperature that last longer than several days and thus describes how acclimation to warmer temperatures can oppose the acute thermodynamic effect of temperature on metabolic rate. We consider our post-acclimation Q_{10} values to be reasonable because we estimated a post-acclimation Q_{10} value of 1.5 for Mel reared in single-species cultures in the present study, which is equivalent to the measured post-acclimation Q_{10} value of 1.5 for Mel, which we calculated from the observed change in mean routine metabolic rate between adults reared and measured at 25 °C and those reared and measured at 30 °C (ref. [27]).

The calculated Q_{10} values were then used to model the responses of metabolic rate to climate warming with and without interspecific interactions at locations where *Drosophila* occur currently. We determined current *Drosophila* locations by downloading occurrence records from GBIF.org using the R package rgbif v.3.7.3 (GBIF Occurrence Download https://doi.org/10.15468/dl.8aymsf, accessed on 17 March 2022)[45]. For each location, we determined recent (1970–2000) and projected future (2081–2100) climates under the SSP 2-4.5 scenario (Coupled Model Intercomparison Project Phase 6, CMIP6) using the mean temperature of the warmest quarter downloaded at a 10 arcmin resolution from WorldClim v.2.1 (ref. [46]) (https://worldclim.org/) using the R package raster v.3.6-3 (ref. [47]) for recent climates and manually downloaded for projected climates. We chose the warmest quarter because we assume that this is when *Drosophila* are most active. Since routine metabolic rate in our study is a mass-independent measure of metabolic rate, we used the interspecific relationship between insect metabolic rate (MR, μW) at 25 °C and mass (m, g) determined by ref. [35] (equation (3)) and a rearrangement of the Q_{10} equation to estimate the routine metabolic rates of *Drosophila* at current temperatures ($MR_{Current}$) in units of μW $g^{-0.75}$ (equation (4)):

$$MR = 10^{3.2} \times m^{0.75} \quad (3)$$

$$MR_{Current} = 10^{3.2} \times Q_{10}^{((T_2-25)/10)} \quad (4)$$

where T_2 is the current temperature and the Q_{10} value used was the acute Q_{10} value of 2.5. We then calculated the changes in temperature (ΔT) at each location between current and future climates for eight global climate models (BCC-CSM2-MR, CanESM5, CNRM-CM6-1, CNRM-ESM2-1, IPSL-CM6A-LR, MIROC-ES2L, MIROC6 and MRI-ESM2-0). For each of the eight climate models we calculated future metabolic rates (MR_{Future}) based on the post-acclimation Q_{10} values calculated for each *Drosophila* species reared in single-species and two-species cultures (Supplementary Table 5) (equation (5)):

$$MR_{Future} = MR_{Current} \times Q_{10}^{(\Delta T/10)} \quad (5)$$

For each of the eight climate models, we calculated the absolute and relative change in metabolic rate between current and future temperatures ($\Delta MR_{Absolute}$ and $\Delta MR_{Relative}$, respectively) (equations (6) and (7), respectively) and also the absolute and relative metabolic cost of interspecific interactions under climate warming ($Cost_{Absolute}$ and $Cost_{Relative}$, respectively) by calculating the difference between future metabolics rates with and without interspecific interactions ($MR_{Future(Comp)}$ and $MR_{Future(Control)}$, respectively) (equations (8) and (9), respectively):

$$\Delta MR_{Absolute} = MR_{Future} - MR_{Current} \quad (6)$$

$$\Delta MR_{Relative} = \left(\frac{MR_{Future} - MR_{Current}}{MR_{Current}}\right) \times 100 \quad (7)$$

$$Cost_{Absolute} = MR_{Future(Comp)} - MR_{Future(Control)} \quad (8)$$

$$Cost_{Relative} = \left(\frac{MR_{Future(Comp)} - MR_{Future(Control)}}{MR_{Future(Control)}}\right) \times 100 \quad (9)$$

We then calculated the mean values for these eight climate models restricting predictions to within 200 km of occurrence localities. We examined the sensitivity of our conclusions to the Q_{10} value used to determine metabolic rates at current temperatures and found that if we use species-specific Q_{10} values that account for acclimation (Q_{10} values <2.5; Supplementary Table 5), the relative costs of interspecific interactions remain unchanged but the absolute costs of interspecific interactions increase by 9–23% for Mel, 4–24% for Sim and 0.4–1.6% for Sulf. By using an acute Q_{10} value of 2.5 to calculate metabolic rates at current temperatures we are therefore presenting conservative estimates of the costs of interspecific interactions under warming.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Drosophila occurrence records were downloaded from https://www.gbif.org/ using the R package rgbif v.3.7.3 (GBIF Occurrence Download https://doi.org/10.15468/dl.8aymsf, accessed on 17 March 2022)[45]. Recent climate data (1970–2000) were downloaded from WorldClim v.2.1 (ref. [46]) (https://worldclim.org/) using the R package raster v.3.6-3 (ref. [47]). Projected future climate data (2081–2100) under the SSP 2-4.5 scenario (CMIP6) for eight global climate models (BCC-CSM2-MR, CanESM5, CNRM-CM6-1, CNRM-ESM2-1, IPSL-CM6A-LR, MIROC-ES2L, MIROC6 and MRI-ESM2-0) were downloaded manually from WorldClim v.2.1 (ref. [46]). All other data generated and analysed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.7475922 (ref. [48]).

Code availability

R code used for data analysis is available in the Zenodo repository, https://doi.org/10.5281/zenodo.7475922 (ref. [48]).

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Author contributions
L.A.A. and V.K. conceived and designed the study, performed the experiments, analysed the data and wrote the paper. L.A.A. performed the modelling.

Competing interests
The authors declare no competing interests.

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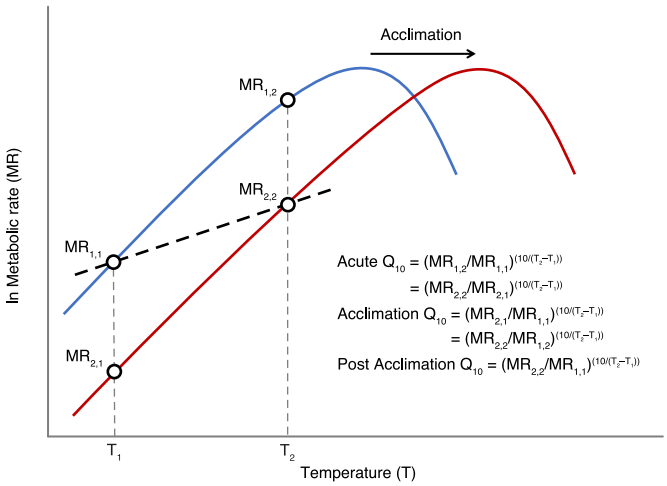
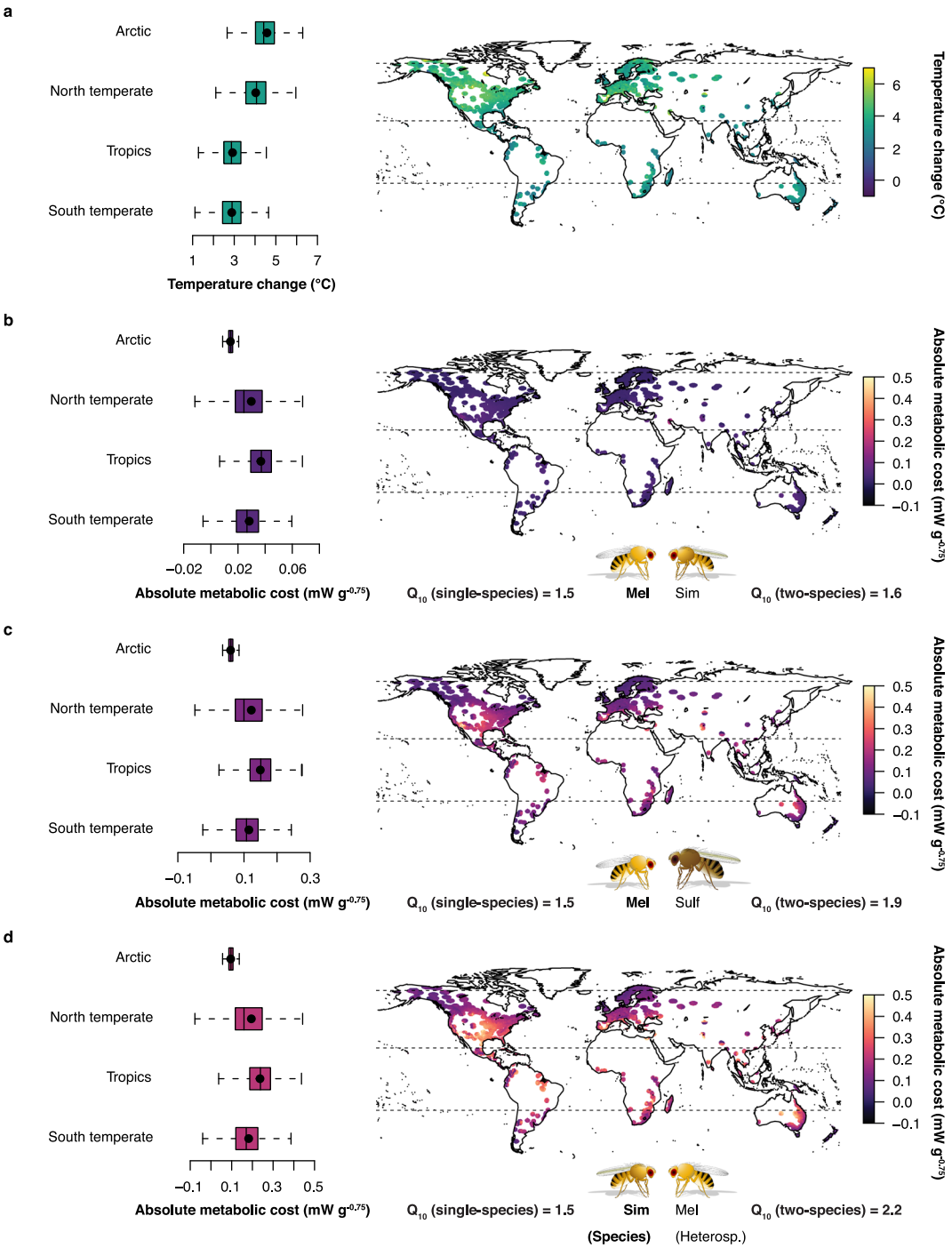


Extended Data Fig. 1 | Conceptual diagram describing the thermal sensitivities of metabolic rate. The thermal sensitivity of metabolic rate can be described by the factorial change in metabolic rate relative to a 10°C change in temperature (Q_{10}). The metabolic rates of ectotherms acclimated to cold (blue line) and warm (red line) temperatures accelerate approximately exponentially with acute increases in temperature as described by the acute Q_{10}. Acclimation to warmer temperatures causes a shift to the right in the reaction norm for metabolic rate. When measured at the same temperature, warm-acclimated ectotherms have lower metabolic rates than cold-acclimated ectotherms as described by the acclimation Q_{10}. When cold- and warm- acclimated ectotherms are measured at their respective acclimation temperatures, the thermal sensitivity of metabolic rate can be described by the post-acclimation Q_{10}. The post-acclimation Q_{10} therefore describes the sensitivity of metabolic rate to changes in temperature that last longer than several days and thus describes how acclimation to warmer temperatures opposes the acute thermodynamic effect of temperature on metabolic rate. The post-acclimation Q_{10} is therefore lower than the acute Q_{10}.

https://doi.org/10.1038/s41558-023-01607-6



Extended Data Fig. 2 | Absolute metabolic cost of interspecific interactions under climate warming. Spatially explicit predictions of the absolute metabolic cost (mW $g^{-0.75}$) of interspecific interactions at the end of the century under an intermediate climate-warming scenario across the global range of *Drosophila* (n = 1,944,000). Changes in temperature (a) at each location were calculated between recent (1970–2000) and projected future (2081–2100) climates using the mean temperature of the warmest quarter at a 10 arcmin resolution. The warmest quarter is assumed to be when *Drosophila* are most active. Future temperatures were extracted from climate projections (CMIP Phase 6) based on eight global climate models (BCC-CSM2-MR, CanESM5, CNRM-CM6-1, CNRM-ESM2-1, IPSL-CM6A-LR, MIROC-ES2L, MIROC6 and MRI-ESM2-0) under the Shared Socio-Economic Pathway 2–4.5 scenario. Predictions are based on the thermal sensitivities of the routine metabolic rate (described by Q_{10} values) of *Drosophila melanogaster* (Mel) (b, c) and *D. simulans* (Sim) (d) (abbreviated name in bold) following developmental thermal acclimation in single-species or two-species cultures with a heterospecific (Heterosp.) (abbreviated name not in bold). For Mel, the heterospecific was either Sim or *D. sulfurigaster* (Sulf). For Sim, the heterospecific was Mel. Metabolic costs of interspecific interactions under climate warming are expressed as the absolute difference between predicted metabolic rates with and without interspecific interactions in units of mW $g^{-0.75}$ (see Eq. 8 in Methods). Predictions are constrained to within 200 km of occurrence localities. Data are summarized for each region with boxes showing the interquartile range (IQR), lines within boxes showing the median, whiskers showing the 1.5×IQR range, and data points are the mean (Arctic: n = 304,560; north temperate: n = 559,440; tropics: n = 604,800; south temperate: 475,200). Outliers are excluded from box plots for visual clarity. Coastline data from *mapdata* v2.3.0[36].

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Reporting Summary

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Drosophila occurrence records were downloaded from GBIF.org using the R package rgbif v3.7.3 (GBIF Occurrence Download https://doi.org/10.15468/dl.8aymsf Accessed on 17 March 2022). Recent climate data (1970–2000) were downloaded from WorldClim v2.1 (worldclim.org) using the R package raster v3.6-3. Projected future climate data (2081–2100) under the Shared Socio-Economic Pathway 2–4.5 scenario (CMIP Phase 6) for eight global climate models (BCC-CSM2-MR, CanESM5, CNRM-CM6-1, CNRM-ESM2-1, IPSL-CM6A-LR, MIROC-ES2L, MIROC6 and MRI-ESM2-0) were downloaded manually from WorldClim v2.1 (worldclim.org). All other data generated and analysed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.7475922.
Data analysis	All data were analysed using R v4.2.0. Generalised linear models were fit using the glmmTMB package v1.14. Linear mixed models were fit using the lme4 package v1.1-30. Full generalised linear mixed models were reduced based on Akaike's information criterion using the bbmle package v1.0.25. Full linear mixed models were reduced using the lmerTest package v3.1-3. The significance of fixed effects in minimum adequate models was tested using the car package v3.1-0. The tests of significance for parameter estimates were performed using the lmerTest package v3.1-3. Model assumptions were tested using the DHARMa package v0.4.5. R code used to analyse data is available in the Zenodo repository, https://doi.org/10.5281/zenodo.7475922.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

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Field-specific reporting

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☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We examined how temperature and interspecific interactions in the developmental environment shape the energy demands of adults of three species of Drosophila (D. melanogaster, D. simulans and D. sulfurigaster). We measured the metabolic rate, activity and body mass of ~400 individual adult virgin female flies (~20 per treatment) that were reared with or without heterospecifics (competition treatment) at one of three mean temperatures (25, 28, or 30°C) (temperature treatment). Treatment effects on adult traits were analysed in models that included an interaction term between the fixed factors of temperature and competition treatment. Metabolic rate data were analysed either with mass and activity, mass only, or without mass and activity as covariates to determine treatment effects on resting, routine, and absolute metabolic rate, respectively. The change in the mean routine metabolic rate associated with developmental thermal acclimation was used to estimate the thermal sensitivity of metabolic rate with and without interspecific interactions which was then used to model the effect of climate warming on metabolic rate.
Research sample	We measured the metabolic rates of three commonly studied species of Drosophila (Drosophila melanogaster, Drosophila simulans and Drosophila sulfurigaster) that originated from stock populations that had been maintained in the laboratory for 8–11 generations. Metabolic rate, activity, and body mass was measured on sexually mature adult virgin females (4–7 days of age) that were reared in the laboratory.
Sampling strategy	Individuals were randomly sampled from treatment vials. No sample size calculations were performed a priori; we measured as many individuals as logistically feasible.
Data collection	Carbon dioxide production (a proxy for metabolic rate) and activity were continuously recorded by machines (gas analysers and activity monitors, respectively) and their corresponding data acquisition software. Body masses were recorded by one person (Lesley Alton). Metabolic rates were calculated as described in the Methods. Viability was measured by counting the number of individuals that emerged (eclosed) from a known number of eggs in each vial per treatment. Viability was recorded by Vanessa Kellermann and Tamblyn Thomason.
Timing and spatial scale	The metabolic rates of flies were measured randomly across a two day period in October 2017.
Data exclusions	Relationships between metabolic rate and body mass and relationships between metabolic rate and activity were assessed visually and data for two flies were excluded as they were clear outliers.
Reproducibility	No attempts were made to repeat the experiment.
Randomization	On selecting the eggs for viability, vials were randomised across all the treatments. During the length of development, fly vials were removed and randomised again to avoid position effects within the temperature-controlled cabinets. For metabolic rate, flies were measured in a randomised order.
Blinding	Prior to metabolic rate measurements, individuals were collected into vials with known treatment. Flies were then placed randomly into numbered chambers that were then connected to the respirometry system. Metabolic rate data from each measurement block were extracted blind to treatment with the data matched to treatment using the corresponding measurement channel.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Flies were collected from the field during the summer months between March and May 2017 and transported back to Monash University in Melbourne, Australia, for initiation of mass bred populations.
Location	Drosophila melanogaster and Drosophila simulans were collected from Melbourne, Australia (37.8136°S, 144.9631°E), and Drosophila sulfurigaster from Cairns, Australia (16.8692°S, 145.6749°E).
Access & import/export	All flies were collected from urban/semi urban environments having minimal impact on native flora and fauna outside the target species. Fewer than 200 flies were removed per site, and given the large population sizes of Drosophila, the impact to populations is likely to be minimal.
Disturbance	Flies were collected via sweep netting over banana baits. Any by catch was released and only the target species was transported back to the laboratory. Given that flies were collected in urban and semi-urban environments, only a small number of flies kept per species, and by-catch was released back into the wild, the impact to the populations and other species is likely to be small.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Our study measured adult virgin female Drosophila melanogaster, Drosophila simulans and Drosophila sulfurigaster that were 4–7 days of age. Flies were obtained from laboratory stock populations that originated from wild collected flies (see Wild Animals below).
Wild animals	Field-inseminated adult females of Drosophila melanogaster and D. simulans were collected from Melbourne, Australia (37.8136°S, 144.9631°E), and D. sulfurigaster were collected from Cairns, Australia (16.8692°S, 145.6749°E), in the summer months between March and May 2017. Flies were collected from urban and semi-urban environments via sweep netting over banana baits. Any by catch was released and only the target species was transported back to Monash University in Melbourne, Australia.
Field-collected samples	Mass-bred populations of Drosophila melanogaster, D. simulans, and D. sulfurigaster were maintained in the laboratory in three bottles containing a yeast-potato-dextrose-agar medium at 25°C under 12:12 h light:dark cycle at an approximate census size of 750–1000. On initiation of the experiment, D. melanogaster had been maintained in the laboratory for 11 generations, while D. simulans and D. sulfurigaster had been maintained for eight generations.
Ethics oversight	No ethical approval was required because work was conducted on insects.

Note that full information on the approval of the study protocol must also be provided in the manuscript.