Developmental nutrition modulates metabolic responses to projected climate change

Lesley A. Alton1 | Teresa C. Kutz1 | Candice L. Bywater1 | Julian E. Beaman1 | Pieter A. Arnold1 | Christen K. Mirth1 | Carla M. Sgro1 | Craig R. White1,2

1School of Biological Sciences, Monash University, Melbourne, Vic, Australia
2Centre for Geometric Biology, Monash University, Melbourne, Vic, Australia

Abstract

1. The current policy has the world on track to experience around 3°C of warming by 2100. The responses of organisms to our warming world will be mediated by changes in physiological processes, including metabolic rate. Metabolic rate represents the energetic cost of living, and is fundamental to understanding the energy required to sustain populations. Current evidence indicates that animals have a limited capacity to adapt to warmer environments by reducing their metabolic rate. Consequently, animals may be more reliant on metabolic plasticity to ameliorate the thermodynamic effect of rising temperatures on physiological rates. However, metabolic plasticity is influenced by other environmental factors, including the nutritional quality of food. Elevated levels of atmospheric CO2 are expected to reduce the protein and increase the carbohydrate concentration in plants, but we do not know how this will affect the response of metabolic rate to climate warming.

2. Here we test the interactive effects of developmental dietary protein and carbohydrate concentrations on the metabolic plasticity of adult Drosophila melanogaster in response to a 3°C increase in temperature while accounting for variation associated with body mass and activity (resting metabolic rate).

3. We show that the thermal sensitivity of resting metabolic rate is modulated by developmental nutrition with animals reared on nutritionally poor, low-protein diets showing the greatest increase in resting metabolic rate in response to simulated climate warming. We also show that if the nutritional quality of resources is unaffected by climate change, then temperature-induced increases in resting metabolic rate will be offset by decreases in mass, but the absolute energy requirements of animals will be elevated relative to current conditions despite this. If, on the other hand, temperatures rise and resources become more calorie-dense and carbohydrate-rich, then the resting metabolic rate of animals will remain relatively unchanged, but decreases in mass and activity may drive down the absolute energy requirements of animals.

4. In the absence of evolutionary adaptation, these findings suggest that the combined plastic response of physiological, morphological and behavioural traits to temperature and nutrition may be an important determinant of the ultimate outcome of climate change for populations.
INTRODUCTION

Global temperatures are expected to rise by around 3°C by the end of the century if current policies remain unchanged (Climate Action Tracker, 2017). Such rapid environmental change threatens biodiversity, with up to one in six species predicted to go extinct as a consequence of climate change (Urban, 2015). To avoid climate change-induced extinction, species must either alter the seasonal phenology of key life-history events (Parmesan & Yohe, 2003), shift their geographic range (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Parmesan & Yohe, 2003; Pecl et al., 2017), alter behavioural patterns (Huey, Hertz, & Sinervo, 2003; Kearney, Shine, & Porter, 2009) or adjust to their new thermal conditions through phenotypic plasticity (Chevin, Lande, & Mace, 2010; Riddell, Odom, Damm, & Sears, 2018) or genetic evolution (Chevin et al., 2010; Hoffmann & Sgrò, 2011). For those species unable to avoid increasing temperatures, their ability to persist will depend on the plasticity and adaptive capacity of physiological traits, such as their metabolic rate (Dillon, Wang, & Huey, 2010; Hoffmann, Hallas, Dean, & Schiffer, 2003; Pörtner & Knust, 2007; Urban et al., 2016). The metabolic rate of an individual is the rate at which it expends energy to survive, forage, grow and reproduce (Brown, Gillooly, Allen, Savage, & West, 2004). Metabolic rate therefore sets the energy demand that organisms exert on their environment, making it central to understanding the flow of energy through ecosystems (Humphries & McCann, 2014; Schramski, Dell, Grady, Sibly, & Brown, 2015). For ectothermic animals, which constitute much of the Earth’s biodiversity, a warming climate is predicted to significantly increase rates of energy expenditure (Dillon et al., 2010) because metabolic rate increases approximately exponentially with acute increases in temperature (Gillooly, Brown, West, Savage, & Charnov, 2001). Krogh’s rule (Gaston et al., 2009; Krogh, 1916) proposes that evolution should oppose this thermodynamic effect of temperature on metabolic rate, such that the energetic cost of living for ectotherms at warmer temperatures should be no more expensive than it is at current temperatures. In other words, Krogh’s rule suggests that ectotherms should exhibit perfect thermal compensation in metabolic rate to maintain their fitness despite changes in temperature. In this instance, when ectotherms from cool and warm environments are measured at the same temperature, those from warmer environments are therefore predicted to exhibit lower metabolic rates than those from cooler environments. Such reductions in metabolic rate in response to elevated temperatures may be an important mechanism by which species cope with climate warming.

Clinal studies comparing species or populations living along latitudinal gradients offer mixed support for Krogh’s rule (Addo-Bediako, Chown, & Gaston, 2002; Berrigan & Patridge, 1997; Galtán-Espitia & Nespolo, 2014; Messamah, Kellermann, Malte, Loeischke, & Overgaard, 2017; Tsuji, 1988; White, Alton, & Frappell, 2012). However, laboratory natural selection across a range of thermal environments results in either no evolved changes in metabolic rate (Alton, Condon, White, & Angilletta, 2017; Berrigan & Patridge, 1997), or increased metabolic rates in warm environments (Mallard, Nolte, Tobler, Kapun, & Schlötterer, 2018). Animals therefore appear to have limited capacity to evolve changes in metabolic rate to oppose the increased energetic cost of living with rising environmental temperatures, even though metabolic rate is significantly heritable for most species (Pettersen, Marshall, & White, 2018). In the absence of evolutionary adaptation in (mean) metabolic rate, phenotypic plasticity is likely to be the major mechanism by which animals alter their rates of energy expenditure (Seebacher, White, & Franklin, 2015).

Plasticity is considered an important mechanism by which species can change their physiological phenotypes to suit new conditions (Seebacher et al., 2015) even though it is not always beneficial (Arnold, Nicotra, & Kruuk, 2019; Duputié, Rutschmann, Ronce, & Chuine, 2015) and may limit the potential for evolutionary (genetic) responses to climate change (Oostra, Saastamoinen, Zwaan, & Wheat, 2018). Indeed, projections of climate-change risk that incorporate physiological plasticity reduce the predicted extinction risk of species substantially (Riddell et al., 2018), though most assessments of risk still do not explicitly include plastic responses (Urban et al., 2016). Elevated temperatures typically cause plastic reductions in metabolic rate, such that, when measured at the same temperature, individuals maintained at higher temperatures have lower metabolic rates (Seebacher et al., 2015). These compensatory responses are similar in outcome as to what is expected under Krogh’s rule, but occur within the lifetime of the organism (acclimation) rather than over evolutionary timescales. Metabolic plasticity may therefore provide animals with a mechanism to counteract the physiological effects of increased temperatures in the absence of adaptive genetic responses. Crucially, however, metabolic rate also exhibits plastic responses to abiotic and biotic variables other than temperature (Burton, Killen, Armstrong, & Metcalfe, 2011; McKechnie, 2008). The response to combinations of stressors may yield sub-optimal phenotypes that are difficult to predict based on single-stressor assessments, and may have important implications for the efficacy of plastic responses as a compensatory mechanism in the face of climate change.

In addition to driving changes in temperature, elevated levels of atmospheric carbon dioxide (CO2) are projected to increase carbohydrates and decrease iron, zinc and protein in plants (Asseng et al., 2019; Lodalze, 2014; Medek, Schwartz, & Myers, 2017; Myers et al., 2014; Zhu et al., 2018), and dietary protein-to-carbohydrate ratios have recently been shown to have plastic effects on metabolic rates (Henry, Overgaard, & Colinet, 2020; Winwood-Smith, Franklin, & White, 2017). Future increases in anthropogenic CO2 emissions may therefore affect organisms not only by causing elevated environmental temperatures, but also by altering the nutritional quality of food sources. The ultimate outcome for organisms challenged by these two
environmental shifts will likely be difficult to predict because the cumulative impact of multiple stressors can be greater or less than the additive combination of the individual effects (Alton & Franklin, 2017; Folt, Chen, Moore, & Burnaford, 1999; Orr et al., 2020). Yet despite the acceptance that stressor interactions are important, and will become increasingly so under climate change, the vast majority of assessments of climate-change risk continue to consider only a single stressor (Kaunisto, Ferguson, & Sinclair, 2016; O’Brien, Dafforn, Charlton, Johnston, & Mayer-Pinto, 2019; Rosenblatt & Schmitz, 2016).

To date, no study has examined the response of metabolic rate to the combined shifts in temperature and nutrition that are expected to occur under climate change. In the present study, we address this knowledge gap by testing the interactive effects of dietary protein and carbohydrate on the metabolic plasticity of adult *Drosophila melanogaster* in response to a 3°C increase in temperature. To do this, we reared flies at either 25°C or 28°C from egg to eclosion (i.e. emergence as an adult) on one of 25 diets that capture the nutrient space that *Drosophila* encounter in nature (Matavelli, Carvalho, Martins, & Mirth, 2015; Silva-Soares, Nogueira-Alves, Beldade, & Mirth, 2017). These diets were a factorial combination of five protein-to-carbohydrate (P:C) ratios (1:8, 1:4, 1:3, 2:3 and 3:2) and five caloric concentrations (0.62, 1.23, 2.46, 4.92 and 9.85 kJ/ml; Figure 1). Following eclosion, adults were maintained at 25°C on a common diet for 6–8 days so that we could attribute any treatment effects to the conditions experienced during larval development rather than to the adult maintenance environment. The rate of CO₂ production of individual adults was then measured at either 25°C or 28°C while they had no access to food and was used as a proxy for metabolic rate. Simultaneous measures of body mass and activity were used to adjust metabolic rates to the sex-specific mean mass and to zero activity, allowing us to examine the effect of developmental diet on the thermal sensitivity of resting metabolic rates independent of treatment effects on body mass and activity.

2 MATERIALS AND METHODS

2.1 Fly stocks

*Drosophila melanogaster* were collected from Ballina, Australia, in April 2016 and mass-bred in the laboratory for approximately 60 discrete generations at a census size of approximately 2,000 individuals (Lasne, Van Heerwaarden, Sgrò, & Connallon, 2019). Stocks were maintained at 25°C and a 12-hr light (L):12-hr dark (D) cycle on a standard yeast-dextrose-potato medium (potato flakes 18.2 g/L; dextrose 27.2 g/L; Brewer’s yeast 36.4 g/L; agar 6.4 g/L; nipagen 10.9 ml/L; and propionic acid 2.3 ml/L).

2.2 Experimental treatments

We designed 25 experimental diets that varied in their protein to carbohydrate (P:C) ratio and caloric concentration according to published protocols (Kutz, Sgrò, Mirth, & Williams, 2019; Rodrigues et al., 2015; Figure 1). Briefly, five P:C ratios (1:8, 1:4, 1:3, 2:3 and 3:2) with a caloric concentration of ~1.23 kJ/ml (assuming 17 kJ/g for protein or carbohydrate, and 37 kJ/g for fat; FAO, 2003) were created by varying the quantities of inactive yeast (containing 45% protein, 33% carbohydrate and 1% fat), dextrose and potato flakes (containing 10% protein and 80% carbohydrate; Table S1). The amount of yeast, dextrose and potato flakes within each ratio was then either halved or multiplied by 2, 4 or 8 to yield five different caloric concentrations per P:C ratio (×0.5 = 0.62 kJ/ml, ×1 = 1.23 kJ/ml, ×2 = 2.46 kJ/ml, ×4 = 4.92 kJ/ml and ×8 = 9.85 kJ/ml).

Experimental flies were obtained by placing parental flies from our fly stocks into egg-laying chambers and allowing them to oviposit overnight on the same yeast-dextrose-potato medium as our fly stocks, but with added blue food dye and double the agar (12.8 g/L). For each experimental diet, 200 eggs were transferred into 10 vials each containing 7 ml of treatment food, with 20 eggs per vial. Five of the 10 replicate vials per diet were placed at 25°C and the other five were placed at 28°C in controlled-temperature cabinets and maintained under a 12-hr L:12-hr D photoperiod (Figure 2). These temperatures were chosen because they represent the average summer temperature currently experienced in south-eastern Australia (25°C treatment) and the 3°C increase in temperature (28°C treatment) projected under climate change for the same region (http://www.bom.gov.au/). Vials were randomly relocated within the cabinet...
twice a day to avoid temperature and light gradient biases. Larvae were left to feed ad libitum until eclosion, and adults were maintained on the standard yeast-dextrose-potato medium at 25°C until measurement (Figure 2). Two days prior to measurement, flies were sexed while knocked down with CO\textsubscript{2} and males and females were returned to separate vials with fresh medium.

### 2.3 Metabolic rate

The rates of CO\textsubscript{2} production ($\dot{V}_{\text{CO}_2}$, $\mu$l/hr) of adult flies were measured as a proxy for metabolic rate using a 14-channel flow-through respirometry (indirect calorimetry) system that expands upon previous systems used for Drosophila (Alton et al., 2017; Arnold, Johnson, & White, 2013; White et al., 2019; Winwood-Smith et al., 2017). Flies were measured inside respirometry chambers that were 65-mm long tubes that were inserted into Trikinetics Drosophila Activity Monitors (DAM) that allowed for simultaneous measurement of activity (note that the tube length available for movement was 45 mm). The rate of CO\textsubscript{2} production and activity of each fly was measured at either 25°C or 28°C for 25 min following a 40-min settling period at the measurement temperature without food (Figure 2). The lowest rate of CO\textsubscript{2} production averaged over 10 min was taken as the measure of metabolic rate for each fly. Activity was taken for the same 10-min period as metabolic rate and equated to the number of times a fly walked past the midpoint of the chamber.
per minute. Immediately following metabolic rate measurements, the mass of flies was determined. Measurements were conducted in a randomized order and conducted over a period of 5 days, and flies were 6–8 days of age at the time of measurement. See Supporting Information for a more detailed description of these methods.

A shortcoming of indirect calorimetry is the reliance on rates of CO₂ production as proxies for metabolic rate. Conversion from rates of CO₂ production to energy metabolism requires knowledge of substrate catabolism, which is often inferred from measurements of respiratory quotient (RQ; the ratio of CO₂ production to O₂ consumption). To check the robustness of our conclusions to larval diet-induced changes in adult substrate catabolism, we explored the potential consequences of the possibility that the substrates that animals catabolize while fasted as adults are influenced by the mixture of substrates (i.e. the P:C ratio) present in their larval diet. To do this, we used a relationship between RQ and dietary P:C ratio derived from the work of Henry et al. (2020) to predict the RQ for the five P:C ratios used in the present study (Figure S1a–c). We then estimated the energy equivalent of CO₂ further details about the conversion from rates of CO₂ production to rates of energy metabolism (i.e. metabolic rates).

To examine the interactive effects of developmental diet and in changes in temperature on absolute rates of CO₂ to examine the interactive effects of developmental diet and in changes in temperature on absolute rates of CO₂ production to rates of energy metabolism (mJ/hr), and re-analysed the data. Throughout the remainder of the methods and results, we will therefore explicitly distinguish between measures of rates of CO₂ production and measures of rates of energy metabolism (i.e. metabolic rates). Further details about the conversion from rates of CO₂ production to rates of energy metabolism are provided in Supporting Information.

### 2.4 Statistical analysis

Data were analysed using linear mixed models (Bates, Mächler, Bolker, & Walker, 2015) in R version 3.6.1 (R Core Team, 2019), with the significance of fixed effects tested using Type III Wald $\chi^2$ tests in the car package (Fox & Weisberg, 2011). The interactive effects of developmental diet (i.e. the linear and quadratic components of protein and carbohydrate concentration), developmental temperature (25 or 28°C) and the acute thermal environment (a measurement temperature of either 25°C or 28°C) on adult VCO₂ and metabolic rate were examined in models that included: (a) a five-way interaction between the fixed factors of sex, the linear component of protein concentration, the linear component of carbohydrate concentration, development temperature and measurement temperature; (b) a four-way interaction between the fixed factors of sex, the quadratic component of protein concentration, development temperature and measurement temperature; (c) a four-way interaction between the fixed factors of sex, the quadratic component of carbohydrate concentration and development temperature; (d) a two-way interaction between the fixed factors of body mass and activity; (e) the fixed factor of age; (f) and random intercepts for measurement channel and measurement block. This model was then simplified using stepwise backwards elimination (Kuznetsova, Brockhoff, & Christensen, 2017) based on Akaike’s information criterion to arrive at a minimum adequate model. The parameter estimates for the minimum adequate model were used to predict the effects of developmental dietary protein and carbohydrate concentrations on VCO₂ and metabolic rate for male and female animals of mean sex-specific mass with zero activity for each of the factor combinations for development and measurement temperature (e.g. Figures 4a, b, d, e and 5a, b, d, e). These adjusted measures of VCO₂ and metabolic rate can therefore be interpreted as mass-independent values for inactive animals, which we will henceforth refer to as resting VCO₂ and resting metabolic rate. The interactive effects of developmental diet and increases in temperature on resting VCO₂ and resting metabolic rate was then examined by calculating the difference between the predicted response surfaces for animals that either developed at 25°C (e.g. Figures 4c and 5c), developed at 28°C (e.g. Figures 4f and 5f), were measured at 25°C (e.g. Figures 4g and 5g) or were measured at 28°C (e.g. Figures 4h and 5h). We also calculated the difference between the predicted response surface of animals that developed and were measured at 28°C and the predicted response surface of animals that developed and were measured at 25°C (e.g. Figures 4i and 5i).

To explore the effect of developmental conditions on body mass, a model was fit to the data that included: (a) a four-way interaction between the fixed factors of sex, the linear component of protein concentration, the linear component of carbohydrate concentration and development temperature; (b) a three-way interaction between the fixed factors of sex, the quadratic component of protein concentration, and development temperature; (c) a three-way interaction between the fixed factors of sex, the quadratic component of carbohydrate concentration and development temperature; (d) the fixed factor of age; (e) a random intercept for measurement block. The full model was then simplified to arrive at a minimum adequate model.

To explore how developmental diet and increases in temperature interact to alter patterns of routine activity in adult flies, we fit and simplified a full model that included the same fixed and random factors (except for the effect of activity) as was used to analyse the VCO₂ data.

Finally, the full model for VCO₂ and metabolic rate was re-fit and simplified with the fixed effects of body mass and activity excluded, to examine the interactive effects of developmental diet and increases in temperature on absolute rates of CO₂ production and energy metabolism inclusive of any effects that the developmental and acute conditions had on body mass and activity. We will henceforth refer to these unadjusted measures of VCO₂ and metabolic rate as absolute VCO₂ and absolute metabolic rate.

### 3 RESULTS

#### 3.1 Comparative analysis of VCO₂ and body mass in *Drosophila melanogaster*

In total, we measured the VCO₂, body mass and activity of 1,909 individual flies giving us a sample size of 7–11 individuals for each of
the 200 factor combinations (two sexes x five P:C ratios x five caloric concentrations x two development temperatures x two measurement temperatures). To assess the reliability of our data, we first calculated the mean \( \dot{V}_{\text{CO}_2} \) and mean mass for each of the 200 factor combinations, and compared them against a database of 53 measurements of \( \dot{V}_{\text{CO}_2} \) and mass for \textit{D. melanogaster} compiled from published studies (see Supporting Information). Our data for \( \dot{V}_{\text{CO}_2} \) and mass fall towards the middle of measurements of \( \dot{V}_{\text{CO}_2} \) published by other research groups (Figure S2) and are consistent with our own previous estimates for this species (Alton et al., 2017; Arnold et al., 2013; Winwood-Smith et al., 2017).

The mean (±standard error, SE) \( \dot{V}_{\text{CO}_2} \) for males ranged from 2.08 ± 0.11 ml/hr to 4.35 ± 0.30 ml/hr, and for females, it ranged from 3.21 ± 0.20 ml/hr to 5.68 ± 0.26 ml/hr (Figures S2 and S8). Across all 200 means, the mean coefficient of variation of \( \dot{V}_{\text{CO}_2} \) (=standard deviation divided by mean) was 0.18 (range 0.07–0.38), which is comparable to that observed for the basal metabolic rate of 624 species of mammal (mean: 0.16, range: 0.002–0.77; Sieg et al., 2009).

### 3.2 Treatment effects on resting \( \dot{V}_{\text{CO}_2} \)

Plots of the relationship between mean resting \( \dot{V}_{\text{CO}_2} \) and developmental diet (P:C ratio x caloric concentration) showed clear non-linearities (Figure 3). The minimum adequate model that explained the observed variation in \( \dot{V}_{\text{CO}_2} \) included significant interactions between: (a) the quadratic component of protein concentration, development temperature and measurement temperature; (b) the linear component of protein concentration, development temperature and measurement temperature; (c) sex and measurement temperature; (d) sex and the quadratic component of carbohydrate concentration; (e) sex and the linear component of carbohydrate concentration; and (f) sex and development temperature (Table 1). In addition to the interactions between these fixed factors on \( \dot{V}_{\text{CO}_2} \), there was also a significant positive effect of mass, and of activity (Table 1). This set of effects reveals sex-specific non-linear effects of developmental dietary protein and carbohydrate concentrations on \( \dot{V}_{\text{CO}_2} \), which differ among development and measurement temperatures (Figures 3–5).

The shapes of the surfaces describing the effect of developmental dietary protein and carbohydrate concentrations on adult resting \( \dot{V}_{\text{CO}_2} \) were different between males and females in that: (a) the strength of the effect of developmental dietary carbohydrate was greater for males than for females and (b) variation in adult resting \( \dot{V}_{\text{CO}_2} \) associated with developmental diet was greater for males than for females (Figures 4a,b,d,e and 5a,b,d,e). For both sexes, the highest resting \( \dot{V}_{\text{CO}_2} \) was observed at an intermediate carbohydrate concentration (4.0–4.4 kJ/ml for males, and 2.6–3.7 kJ/ml for females), irrespective of development and measurement temperature. The response of adult resting \( \dot{V}_{\text{CO}_2} \) to developmental dietary protein concentration was similar for both sexes but varied among development and measurement temperature combinations. For animals that developed and were measured at 25°C, an intermediate protein concentration (2.1 kJ/ml) resulted in the highest resting \( \dot{V}_{\text{CO}_2} \) (Figures 4a and 5a). For all other development and measurement combinations, the highest resting \( \dot{V}_{\text{CO}_2} \) was observed at low protein concentrations (0.3–0.6 kJ/ml), though for animals that developed at 28°C and were measured at 25°C, resting \( \dot{V}_{\text{CO}_2} \) was also relatively high at the highest protein concentration (5.5 kJ/ml; Figures 4b and 5b).

For males, the lowest resting \( \dot{V}_{\text{CO}_2} \) was observed at either the lowest or highest carbohydrate concentrations when protein concentrations were below 1.1 kJ/ml. Low male resting \( \dot{V}_{\text{CO}_2} \) was also observed at the highest protein concentration (5.5 kJ/ml) with the exception being those that developed at 28°C and were measured at 25°C. In females, the lowest resting \( \dot{V}_{\text{CO}_2} \) was generally observed at the highest protein concentration (5.5 kJ/ml). However, for females that developed at 28°C and were measured at 25°C, the lowest resting \( \dot{V}_{\text{CO}_2} \) was observed at a high carbohydrate (8 kJ/ml) and low-to-intermediate protein (1.8 kJ/ml) concentration. As such, for males, developmental protein and carbohydrate concentrations were similar in their contributions to variation in adult resting \( \dot{V}_{\text{CO}_2} \) with the greatest relative difference from the lowest to the highest resting \( \dot{V}_{\text{CO}_2} \) being 14%–19%. In contrast, the greatest relative difference from the lowest to the highest resting \( \dot{V}_{\text{CO}_2} \) in females was 5%–11% and was predominantly associated with developmental protein concentration. The exception to these patterns was for those flies that developed at 28°C and were measured at 25°C where variation in resting \( \dot{V}_{\text{CO}_2} \) was greatest along the carbohydrate axis for both males and females.

Changes in adult resting \( \dot{V}_{\text{CO}_2} \) associated with a 3°C rise in temperature were strongly affected by developmental dietary protein, development temperature and measurement temperature for both sexes. For animals that developed at 25°C, the relative change in resting \( \dot{V}_{\text{CO}_2} \) associated with an acute 3°C rise in temperature ranged from 5% to 15% for males and from 6% to 12% for females with the acute thermal sensitivity of resting \( \dot{V}_{\text{CO}_2} \), being lowest at an intermediate protein concentration (3 kJ/ml) and greatest at the lowest protein concentration (0.08 kJ/ml; Figures 4g and 5g). For animals that developed at 28°C, the relative change in resting \( \dot{V}_{\text{CO}_2} \) associated with an acute 3°C rise in temperature ranged from −1% to +15% for males and from 3% to 14% for females with the acute thermal sensitivity of resting \( \dot{V}_{\text{CO}_2} \) being lowest at the highest protein concentration (5.5 kJ/ml) and greatest at an intermediate protein concentration (2 kJ/ml; Figures 4h and 5h).

Developmental acclimation to a warmer temperature predominantly caused a reduction in the resting \( \dot{V}_{\text{CO}_2} \) of both sexes at both measurement temperatures. For animals that were measured at 25°C, the relative change in resting \( \dot{V}_{\text{CO}_2} \) associated with developmental acclimation to 28°C ranged from ~8% to ~13% for males and from ~9% to ~4% for females with the greatest negative relative changes occurring at an intermediate protein concentration (2.4 kJ/ml) and positive relative changes occurring only at protein concentrations above 5 kJ/ml (Figures 4c and 5c). For animals that were measured at 28°C, the relative changes in resting \( \dot{V}_{\text{CO}_2} \), associated with developmental acclimation to 28°C were not as substantial (from ~0.4% to +0.9% for males and ~4% to ~3% for females), and the pattern of the response to protein was reversed compared to those measured at 25°C; intermediate protein concentrations
resulted in small positive relative changes in males and small negative relative changes in females, and the greatest negative relative change occurred at the lowest protein concentration (0.08 kJ/ml) for both sexes (Figures 4f and 5f). Developmental acclimation to 28°C reduced but did not eliminate the thermal sensitivity of resting $V_{CO_2}$ to a 3°C increase in temperature: males and females that developed and were measured at 28°C had 6%–15% and 4%–8% higher resting $V_{CO_2}$ than males and females that developed and were measured at 25°C respectively. The post-acclimation thermal sensitivity of resting $V_{CO_2}$ was lowest at an intermediate protein concentration (3 kJ/ml).
and greatest at the lowest protein concentration (0.08 kJ/ml) for both sexes (Figures 4i and 5i).

### 3.3 Treatment effects on resting metabolic rate

The shapes of the surfaces describing the effect of developmental dietary protein and carbohydrate concentrations on adult metabolic rate were different in comparison to those for \( V_{\text{CO}_2} \) such that peaks in resting metabolic rate was generally shifted towards a lower carbohydrate and a higher protein concentration for both males (Figure S3a,b,d,e) and females (Figure S4a,b,d,e). However, the minimum adequate model that explained the observed variation in metabolic rate included the same significant interactions as those for \( V_{\text{CO}_2} \) (Table S2). Consequently, the response patterns to developmental diet and a 3°C increase in temperature were the same for resting \( V_{\text{CO}_2} \) (Figures S3c,f–i) prompting no changes to our overall conclusions.

### 3.4 Treatment effects on adult body mass and routine adult activity

The minimum adequate model that explained the observed variation in body mass indicated there were significant sex-specific nonlinear effects of developmental dietary protein and carbohydrate concentration (Table S3). The shapes of the surfaces describing the effect of developmental dietary protein and carbohydrate concentration on adult body mass were different between males and females in that the strength of the effect of developmental dietary protein was greater for females (Figure S5c,d) than for males (Figure S5a,b). A developmental diet with an intermediate protein and carbohydrate concentration produced the largest adults for both sexes, although this occurred at slightly lower protein and carbohydrate concentrations for females (2.4 and 2.2 kJ/ml, respectively) compared to males (2.9 and 2.9 kJ/ml, respectively). For both sexes, a developmental diet with a low protein (1.1 kJ/ml) and high carbohydrate (8.7 kJ/ml) concentration produced the smallest adults, with the relative difference in body mass from the smallest to the largest adults being 31%. The minimum adequate model for body mass also revealed that development at 28°C caused a significant ~5% reduction in adult body mass irrespective of sex and developmental diet (Table S3).

The minimum adequate model for routine activity revealed that variation in adult routine activity was explained only by sex, body mass and developmental dietary carbohydrate concentrations (Table S4); males were more active than females, smaller animals were more active than larger animals, and animals that developed on high carbohydrate diets were 10%–14% less active as adults than those that developed on low carbohydrate diets (Figures S6 and S7). Neither the temperature experienced during development, nor the

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<td>1</td>
<td>0.262</td>
</tr>
<tr>
<td>Measurement temperature</td>
<td>0.4965</td>
<td>0.2830</td>
<td>3.08</td>
<td>1</td>
<td>0.079</td>
</tr>
<tr>
<td>Sex (Male) × Carbohydrate</td>
<td>0.1264</td>
<td>0.0407</td>
<td>9.63</td>
<td>1</td>
<td>0.002**</td>
</tr>
<tr>
<td>Sex (Male) × Carbohydrate(^2)</td>
<td>−0.0138</td>
<td>0.0048</td>
<td>8.11</td>
<td>1</td>
<td>0.004**</td>
</tr>
<tr>
<td>Sex (Male) × Meas. temp.</td>
<td>−0.0470</td>
<td>0.0183</td>
<td>6.59</td>
<td>1</td>
<td>0.010*</td>
</tr>
<tr>
<td>Protein × Dev. temp.</td>
<td>−0.8957</td>
<td>0.3761</td>
<td>5.67</td>
<td>1</td>
<td>0.017*</td>
</tr>
<tr>
<td>Protein × Meas. temp.</td>
<td>−0.8604</td>
<td>0.3763</td>
<td>5.23</td>
<td>1</td>
<td>0.022*</td>
</tr>
<tr>
<td>Protein(^2) × Dev. temp.</td>
<td>0.1846</td>
<td>0.0755</td>
<td>5.97</td>
<td>1</td>
<td>0.015*</td>
</tr>
<tr>
<td>Protein(^2) × Meas. temp.</td>
<td>0.1751</td>
<td>0.0755</td>
<td>5.37</td>
<td>1</td>
<td>0.020*</td>
</tr>
<tr>
<td>Dev. temp. × Meas. temp.</td>
<td>−0.0132</td>
<td>0.0107</td>
<td>1.53</td>
<td>1</td>
<td>0.215</td>
</tr>
<tr>
<td>Protein × Dev. temp. × Meas. temp.</td>
<td>0.0323</td>
<td>0.0142</td>
<td>5.19</td>
<td>1</td>
<td>0.023*</td>
</tr>
<tr>
<td>Protein(^2) × Dev. temp. × Meas. temp.</td>
<td>−0.0066</td>
<td>0.0028</td>
<td>5.45</td>
<td>1</td>
<td>0.020*</td>
</tr>
</tbody>
</table>

\( p < 0.05; \quad **p < 0.01; \quad ***p < 0.001.\)
FIGURE 4  The predicted interactive effects of developmental diet, developmental temperature and the acute thermal environment on the resting rates of CO\textsubscript{2} production (\(\dot{V}_{\text{CO}_2}\), µl/hr) of adult male Drosophila melanogaster. Panels with the white background (a, b, d and e) show the predicted effects of developmental dietary protein and carbohydrate concentrations (kJ/ml) on the resting \(\dot{V}_{\text{CO}_2}\) of males that developed at either 25°C or 28°C (T\text{development}) and were measured at either 25°C or 28°C (T\text{measurement}). Panels with the grey background (c, f, g, h and i) show the differences in resting \(\dot{V}_{\text{CO}_2}\) when comparing among panels with the white background: panels c and f show the differences in resting \(\dot{V}_{\text{CO}_2}\) associated with developing at a warmer temperature for males measured at either 25°C (panel c = panel b minus panel a) or 28°C (panel f = panel e minus panel d); panels g and h show the changes in resting \(\dot{V}_{\text{CO}_2}\) associated with an acute 3°C increase in temperature for males reared at either 25°C (panel g = panel d minus panel a) or 28°C (panel h = panel e minus panel b); and panel i shows the differences in resting \(\dot{V}_{\text{CO}_2}\) between males that developed and were measured at 28°C compared to those that developed and were measured at 25°C (panel e minus panel a). Data shown are the predicted resting \(\dot{V}_{\text{CO}_2}\) values for an inactive adult male fly weighing 0.76 mg (mean male mass) based on the parameter estimates for the minimum adequate model in Table 1. Warmer colours represent higher values and the contours indicate predicted resting \(\dot{V}_{\text{CO}_2}\) values (panels a, b, d and e) or differences in resting \(\dot{V}_{\text{CO}_2}\) (panels c, f, g, h and i). Data are adjusted for the random intercepts of measurement block and measurement channel. Open circles represent the nutritional coordinates of each of the 25 diets used and shown in Figure 1.
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FIGURE 5 The predicted interactive effects of developmental diet, developmental temperature and the acute thermal environment on the resting rates of CO$_2$ production ($\dot{V}$CO$_2$, $\mu$l/hr) of adult female Drosophila melanogaster. Panels with the white background (a, b, d and e) show the predicted effects of developmental dietary protein and carbohydrate concentrations (kJ/ml) on the resting $\dot{V}$CO$_2$ of females that developed at either 25°C or 28°C ($T_{\text{development}}$) and were measured at either 25°C or 28°C ($T_{\text{measurement}}$). Panels with the grey background (c, f, g, h and i) show the differences in resting $\dot{V}$CO$_2$ when comparing among panels with the white background: panels c and f show the differences in resting $\dot{V}$CO$_2$ associated with developing at a warmer temperature for females measured at either 25°C (panel c = panel b minus panel a) or 28°C (panel f = panel e minus panel d); panels g and h show the changes in resting $\dot{V}$CO$_2$ associated with an acute 3°C increase in temperature for females reared at either 25°C (panel g = panel d minus panel a) or 28°C (panel h = panel e minus panel b); and panel i shows the differences in resting $\dot{V}$CO$_2$ between females that developed and were measured at 28°C compared to those that developed and were measured at 25°C (panel e minus panel a). Data shown are the predicted resting $\dot{V}$CO$_2$ values for an inactive adult female fly weighing 1.19 mg (mean female mass) based on the parameter estimates for the minimum adequate model in Table 1. Warmer colours represent higher values and the contours indicate predicted resting $\dot{V}$CO$_2$ values (panels a, b, d and e) or differences in resting $\dot{V}$CO$_2$ (panels c, f, g, h and i). Data are adjusted for the random intercepts of measurement block and measurement channel. Open circles represent the nutritional coordinates of each of the 25 diets used and shown in Figure 1.
acute thermal environment caused any significant changes in adult routine activity levels (Table S4; Figures S6 and S7).

3.5 | Treatment effects on absolute $V_{CO_2}$ and absolute metabolic rate

When body mass and activity were excluded from the full model fit to either the $V_{CO_2}$ or metabolic rate data, the resulting minimum adequate models no longer included significant interaction terms between factors related to diet or temperature. The minimum adequate models were the same for $V_{CO_2}$ and metabolic rate (Tables S5 and S6). Consequently, the shapes of the surfaces describing the effect of developmental dietary protein and carbohydrate concentrations on absolute $V_{CO_2}$ and metabolic rate were similar at each development and measurement temperature combination, and the thermal sensitivity of absolute rates to a 3°C increase in temperature did not vary with the macronutrient composition of the developmental diet (Figures S9–S12).

As with resting rates, the shapes of the surfaces describing the effect of developmental dietary protein and carbohydrate concentrations on adult absolute $V_{CO_2}$ and metabolic rate were different between males and females, in that (a) the strength of the effect of developmental dietary carbohydrate was greater for males than for females and (b) variation in absolute rates associated with developmental diet was greater for males than for females. However, for both sexes, a developmental diet with an intermediate protein (2–2.8 kJ/ml) and low-to-intermediate carbohydrate concentration (1.9–3.6 kJ/ml) led to the highest absolute rates in adults, whereas a developmental diet with a low protein (1.1 kJ/ml) and high carbohydrate (8.7 kJ/ml) concentration led to the lowest absolute rates. The relative difference from the lowest to the highest absolute rates was 35% and 46% for male absolute $V_{CO_2}$ and metabolic rate, respectively, whereas in females the differences were smaller and were 21% and 30% for female absolute $V_{CO_2}$ and female metabolic rate respectively.

Developmental acclimation to 28°C caused a significant decrease in absolute rates (~4% for males, ~6% for females), while acute exposure to 28°C led to a significant increase in absolute rates (+9% for males, +10% for females), with these responses to temperature being significantly weaker in males than in females. Developmental acclimation to 28°C reduced but did not eliminate the thermal sensitivity of absolute rates to a 3°C increase in temperature: males and females that developed and were measured at 28°C had 5% and 3% higher absolute rates than males and females that developed and were measured at 25°C respectively.

4 | DISCUSSION

Recent climate warming has been estimated to cause significant increases in rates of energy expenditure for ectothermic animals (Dillon et al., 2010), and laboratory natural selection experiments suggest that animals have limited evolutionary capacity to counteract this effect (Alton et al., 2017; Berrigan & Patridge, 1997; Mallard et al., 2018). In the absence of evolutionary adaptation, phenotypic plasticity is the major avenue by which animals might adjust their physiology to cope with climate warming (Chevin, Collins, & Lefèvre, 2013; Seebacher et al., 2015). Given that climate change is also predicted to alter plant nutrient content (Rosenblatt & Schmitz, 2016), it is important to examine the plastic responses of animals to both these challenges simultaneously. Here we present the first comprehensive analysis of the interactive effects of developmental diet and increases in temperature on the thermal sensitivity of metabolic rate in adults of an insect model, D. melanogaster. We assume that the rate of $CO_2$ production of a fasted animal is a valid proxy for metabolic rate in the present study because adults were maintained from eclosion on a common diet for 6–8 days prior to measurement, during which, they had no access to food. We therefore refer to rate of $CO_2$ production as metabolic rate throughout the remainder of the discussion, and verify that violation of this assumption does not alter our major conclusion in the results and Supporting Information.

In agreement with previous work, our results show that acclimation to a warmer temperature does not result in perfect thermal compensation of resting rates of energy expenditure (Seebacher et al., 2015). Rather, temperature-induced developmental plasticity only partially opposes the increase in resting metabolic rate (metabolic rate adjusted to mass-independent values for inactive animals) that occurs in response to simulated climate warming (Figures 4i and 5i). Our research, however, is the first to demonstrate that developmental nutrition is a powerful modulator of the thermal sensitivity of resting metabolic rate in adults. Individuals reared on the lowest protein diets showed the least capacity for compensatory developmental plasticity and therefore experienced the strongest effects of temperature on resting rates of energy expenditure compared to those reared on an intermediate protein diet (Figures 4i and 5i). These conclusions are not altered if we assume that the substrates being catabolized during adult respirometry trials are influenced by developmental diet (Figures S3i and S4i). However, it would be valuable for future studies to examine this experimentally by measuring the energy equivalents of $O_2$ and $CO_2$ using simultaneous direct and indirect calorimetry (Walsberg & Hoffman, 2005, 2006), and to examine the effect of diet on mitochondrial function and the relationship between $O_2$ consumption and ATP production (Salin, Auer, Rey, Selman, & Metcalfe, 2015).

Interestingly, the developmental protein concentrations that minimize the thermal sensitivity of resting metabolic rates correspond with the range of developmental protein concentrations that optimize fitness-related life-history traits in D. melanogaster maintained at 25°C. Specifically, diets with intermediate protein concentrations between 0.85 kJ/ml (50 g/L) and 3.4 kJ/ml (200 g/L), and carbohydrate concentrations below 3.4 kJ/ml (200 g/L) are the most consistent at promoting the fastest larval development rates, best larval survival, highest adult female ovariole number, and largest adult body size and mass (Gray, Simpson, & Polak, 2018; Kutz et al., 2019; Rodrigues et al., 2015). Consistent with these findings,
we found that developmental macronutrient concentrations within these ranges produced the largest adults (Figure S5). With elevated levels of atmospheric CO$_2$ projected to decrease the protein and increase the carbohydrate concentrations in plants (Asseng et al., 2019; Loladze, 2014; Medek et al., 2017; Myers et al., 2014; Zhu et al., 2018), the macronutrient composition of resources for developing D. melanogaster may become less favourable in the future. This may be particularly so, given that warmer temperatures have been shown to reduce the nutrient space that optimizes trait outcomes for larval D. melanogaster, as well as shift it towards more protein-rich diets in the case of development time (Kutz et al., 2019). As such, if temperatures rise by 3°C and the protein concentration of the available nutritional resources for developing D. melanogaster drops below 0.85 kJ/ml, then our data suggest that the resting rates of energy expenditure in adults will be significantly higher than if they were only challenged by increases in temperature.

Given that our measure of resting metabolic rate is independent of treatment effects on body mass and activity, our data suggest that the mass-independent energy requirements for self-maintenance (i.e. tissue and internal homeostasis maintenance) will likely be greater in a warmer world, and greater still if nutritional resources become protein-poor. However, we also show that developmental diet and increases in temperature have profound effects on adult body mass and routine activity levels. Development at a warmer temperature significantly reduces adult mass, with the decrease in mass being more pronounced if animals are also reared on high-calorie, carbohydrate-rich diets (Figure S5). Adult routine activity on the other hand is not influenced by temperature but is also significantly lower if animals are reared on high-calorie, carbohydrate-rich diets (Figures S6 and S7). The cumulative impact of rising temperatures and changes in nutritional stoichiometry on rates of energy expenditure in animals therefore depends on the combined responses of physiological, morphological and behavioural traits. We examined this by analysing our metabolic rate data without accounting for variation associated with body mass and activity, which we have termed as absolute metabolic rate (Figures S9–S12). As with resting metabolic rate, the macronutrient composition of the developmental diet causes substantial variation in absolute metabolic rate. Similarly, developmental acclimation to a warmer temperature only partially opposes the increase in absolute metabolic rate associated with a 3°C rise in temperature, but the extent of the increase is smaller in comparison to resting metabolic rate (5% vs. 6%–15% in males, and 3% vs. 4%–8% in females), and does not vary with developmental diet. It therefore seems that the temperature-induced increase in mass-independent resting metabolic rate observed across the entire nutrient space (Figures 4i and 5i) is partially offset by a temperature-induced reduction in body mass (~5% for both sexes; Figure S5). It also seems that decreases in absolute metabolic rate associated with a simultaneous increase in temperature and shift from a protein-rich diet with intermediate calories to a high-calorie, carbohydrate-rich diet (Figure S9a vs. S9d and Figure S10a vs. S10d) is driven predominantly by reductions in body mass (Figure S5a vs. S5b and Figure S5c vs. S5d) and activity (Figure S6a vs. S6d and Figure S7a vs. S7d), rather than a change in resting metabolic rate, which increases slightly in females and remains similar in males (Figure 4a vs. 4e and Figure 5a vs. 5e).

Our finding that temperature and developmental diet interact to cause correlated responses in adult metabolic rate, body mass and activity has potential implications for the consequences of climate change for energy allocation towards other fitness-related traits such as reproductive success, and thus animal abundance and population persistence. Our data add further experimental support to the consensus that warmer temperatures will lead to higher metabolic rates (in terms of both resting and absolute rates) in the absence of the capacity to evolve reductions in metabolic rate to cope with increasing temperatures. If, however, animals are simultaneously faced with nutritionally poor food resources, then our study indicates that maintenance costs may be even greater, or body masses and activity may be even lower. Such changes may lead to lower reproductive success because either higher maintenance costs trade-off against energy allocation towards reproduction (as per the allocation model; Careau, Thomas, Humphries, & Réale, 2008; but see Burton et al., 2011), changed behaviour leads to fewer successful encounters with mates, or smaller body mass leads to fewer offspring (but see Robertson, 1957; Berrigan, 1991 who showed that temperature-induced changes in mass yield no changes in lifetime fecundity; Nunney & Cheung, 1997). It will be difficult to integrate these complex effects, but they warrant further consideration, particularly in an evolutionary framework. If animals are unable to evolve reductions in metabolic rate to cope with the multiple challenges presented by climate change, and if they also have limited capacity to respond through phenotypic plasticity (Figures 4i and 5i), then populations may be at increased risk of extinction. This is because extinction risk is negatively related to carrying capacity (Lande, 1993), which is inversely proportional to the metabolic rate of the individuals in a population (Damuth, 1981; Savage, Gillooly, Brown, West, & Charnov, 2004), such that a given environment can support fewer animals of higher metabolic rate.

The ultimate effect of climate change on populations will therefore depend on the net outcome of changes in habitat productivity (Chu et al., 2016; Meillio et al., 1993; Nemani et al., 2003) together with habitat degradation and fragmentation, and the effects of temperature and diet on life-history traits that require substantial energetic investment such as maintenance, locomotion, growth and reproduction. If energy availability in the environment increases less than metabolic rate, then carrying capacity will decrease and populations will be at increased risk of extinction due to environmental or demographic stochasticity (Lande, 1993). Future work should explore this issue in single- and multi-species mesocosm settings, to gain a more complete understanding of the interactive effects of temperature, dietary macronutrient composition, food availability and within- and among-species interactions on individual physiology and life history, and population and community density, stability and persistence.
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AUTHORS’ CONTRIBUTIONS

L.A.A., T.C.K., C.K.M., C.M.S. and C.R.W. designed the study; L.A.A., T.C.K., P.A.A. and J.E.B. maintained the animals; L.A.A., C.L.B., P.A.A., J.E.B. and C.R.W. collected the data; C.L.B. extracted the data; L.A.A. and C.R.W. analysed the data; L.A.A., J.E.B. and C.R.W. and wrote the first version of the manuscript. All authors contributed to and approved the final version.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.rbnzs7h90 (Alton et al., 2020).

ORCID

Lesley A. Alton https://orcid.org/0000-0002-4236-2494
Teresa C. Kutz https://orcid.org/0000-0003-0287-082X
Pieter A. Arnold https://orcid.org/0000-0002-6158-7752
Christen K. Mirth https://orcid.org/0000-0002-9765-4021
Carla M. Sgro https://orcid.org/0000-0001-7950-2246
Craig R. White https://orcid.org/0000-0002-0200-2187

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.