Physiological traits and their relationships vary along an aridity gradient within and among Fijian bee species

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Abstract

Temperature and water availability are independently hypothesised to be important abiotic drivers of the evolution of metabolic rates and gas exchange patterns, respectively. Specifically, the metabolic cold adaptation hypothesis (MCA) predicts that cold environments select for faster metabolic rates to counter the thermodynamics of biochemical reactions while the hygric hypothesis predicts that dry environments select for discontinuous gas exchange to reduce water loss. Although these two hypotheses consider different physiological traits and how they vary along different abiotic gradients, metabolic rate drives gas exchange patterns in insects meaning these two traits are inherently linked. Despite this link, the MCA and hygric hypotheses are rarely considered together and the extent to which metabolic rates and respiratory patterns vary and co-vary with temperature and aridity along climatic gradients remains unclear. We tested the MCA and hygric hypotheses within a species of endemic Fijian bee, *Homalictus fijiensis*, across an altitudinal gradient of 1100 m, and among four Fijian bee species, including *H. fijiensis*, that inhabit different altitudinal bands. In Fiji, environmental temperature is ~5°C lower in the central highlands than in the coastal lowlands with the highlands receiving ~100 mm of additional precipitation than the lowlands each month. We found an MCA-like pattern within *H. fijiensis* and among Fijian bee species, where metabolic rate decreased with increasing temperature, but precipitation also explained variation in metabolic rate. We also found support for the hygric hypothesis within *H. fijiensis* and across species. Lastly, we found that the relationship between metabolic rate and ventilation rate changed depending on precipitation of the driest month within *H. fijiensis* and among bee species. Bees that inhabited wet regions had low ventilation rates of greater volume but bees that inhabited drier locations had rapid ventilations of smaller volume potentially as a mechanism to exchange gas without desiccating. As the correlation between metabolic rate and ventilation rate changes across the precipitation gradient, it is possible that these two traits can evolve independently of each other in response to abiotic variables despite being positively correlated.

Introduction

Variation in climate shapes species' physiological traits and geographic distributions [1–3]. For terrestrial ectotherms in particular, environmental temperature and aridity are hypothesised to be important abiotic drivers of the evolution of key physiological traits such as metabolic rate and respiratory patterns [4,5]. These two traits underpin the adaptive hypotheses known as the metabolic cold adaptation (MCA) and hygric hypotheses, which aim to explain how and why metabolic rate and respiratory patterns, respectively, vary among populations and species that occupy different climatic niches [5–10]. However, studies that seek to understand the environmental drivers of variation in these two traits rarely consider the MCA and hygric hypotheses together (but see [9,11]), despite the knowledge that increases in metabolic rate result in higher rates of ventilation [12–14]. Given the inherent link between metabolic rate and respiratory pattern, it is plausible that these two traits have co-evolved meaning that selection on metabolic rate has potentially influenced respiratory patterns, and vice versa. However, even though these traits are linked, selection could be acting on each trait independently, causing a change in the relationship between metabolic rate and ventilation rate across an environmental gradient. Thus, to better understand the evolution of one trait requires consideration of the other to determine how environments have shaped trait variation and covariation.

The metabolic cold adaptation hypothesis predicts that organisms from cold environments (such as high latitudes and altitudes) will have higher metabolic rates than those from warm environments, when measured at the same temperature. This pattern is expected to arise as a mechanism to maintain physiological function in cold environments because acute exposure to colder temperatures causes biochemical reaction rates to slow [5,6,15,16]. However, in terrestrial organisms, the pattern described by the MCA hypothesis may also emerge as a consequence of organisms depressing their metabolic rate in response to high temperatures to prevent desiccation [17]. This is because high air temperatures result in high vapour pressure deficits (VPDs) that can lead to higher rates of water loss from wet respiratory surfaces [10]. Organisms living in warmer environments that are also more desiccating could therefore slow their metabolic rates, reduce their ventilation rate, and breathe discontinuously (that is, alternate between periods of ventilation and breath-holding).

The hygric hypothesis makes the explicit prediction that discontinuous gas exchange in resting tracheate arthropods, such as insects, is an adaptation to limit respiratory water loss. As such, drier environments are expected to favour arthropods that breathe discontinuously, and by extension, those that hold their breath for longer (i.e., have a lower ventilation rate) [4,9,18].

In insects, both the MCA and hygric hypotheses tend to be supported by broad-scale comparative analyses [6,18], but not always [19]). In contrast, experimental evolution studies have been unable to replicate the macrophysiological pattern described by the MCA hypothesis by manipulating temperature in controlled laboratory environments [7,20]. Such research indicates that temperature alone may not be responsible for driving the evolution of metabolic rate. The hygric hypothesis similarly suffers from equivocal support [21], and alternative adaptive hypotheses propose that discontinuous gas exchange has evolved as a mechanism to improve gas exchange in underground environments, or to reduce oxidative tissue damage [8,22–24]. It has also been suggested that discontinuous gas exchange is not an adaptation to environmental conditions, but instead arises as a consequence of neural control of gas exchange during rest [25,26]. Given that there is mixed support for both the MCA and hygric hypotheses, and that selection rarely acts on single traits independently [27–29], we propose that these hypotheses should be considered simultaneously to better understand how metabolic rate and respiratory patterns vary and co-vary along climatic gradients.

Here we examine how metabolic rate and respiratory patterns vary and co-vary within and among bee species that breathe discontinuously [30] and exist along an altitudinal gradient on the tropical mountainous island of Viti Levu, Fiji. Altitudinal gradients are powerful for testing hypotheses concerning climatic drivers of trait variation because the spatial rate of change in climate is greater along altitudinal gradients than latitudinal gradients, while habitat composition and photoperiod vary less [31]. Viti Levu is divided by a mountain range that runs north to south and the highest peak is 1328 m above sea level (a.s.l). The highlands in the centre of the island are characterised by cloud forests that are ~ 5°C cooler and receive ~100 mm less precipitation per month than the coastal lowlands (Fig. 1). Due to the mountain range that divides Viti Levu, there is also a rain shadow on the western side of the island that

receives significantly less rainfall than the eastern side (Fig. 1c). The bee species that inhabit Viti Levu occupy different altitudinal ranges: the endemic *Homalictus fijiensis*, Perkins & Cheesman (1928), is found along the entire altitudinal gradient while 22 other endemic *Homalictus* species are restricted to the highlands, and the invasive *Braunsapis puangensis*, Cockerell (1928), is generally restricted to the lowlands [32–34].

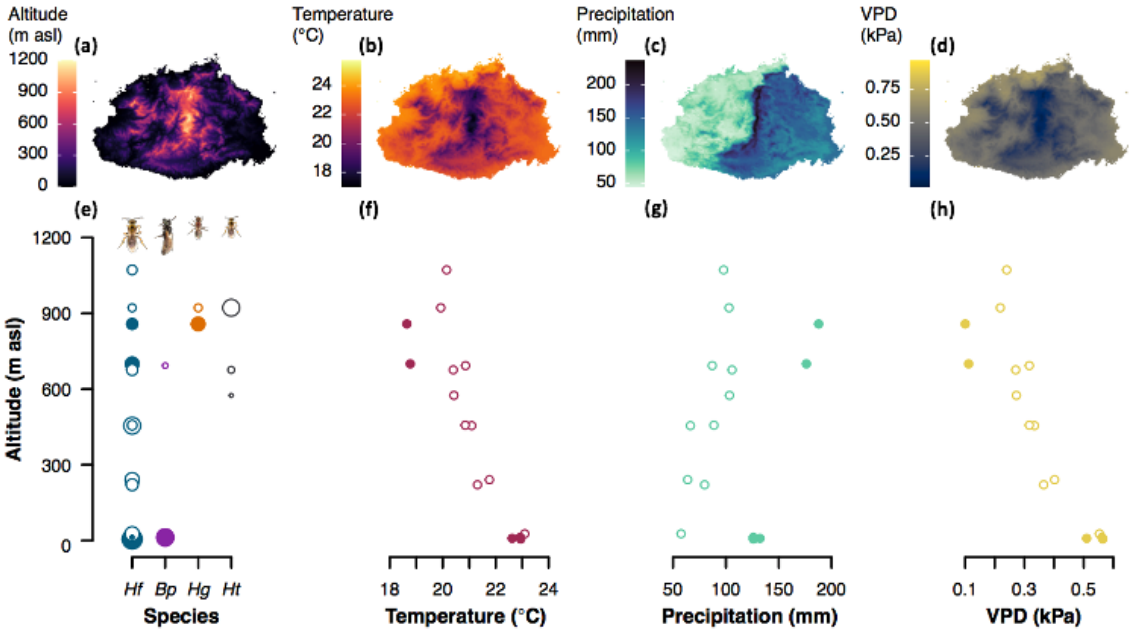

Figure 1. Abiotic variables across Viti Levu, Fiji: a) altitude (m asl), b) temperature of the coolest and driest month (July) (°C), c) precipitation of the driest month (July) (mm) and, d) vapour pressure deficit (July) (kPa). e) Collection altitude of each species (Hf: *Homalictus fijiensis*, Bp: *Braunsapis puangensis*, Hg: *Homalictus groomi*, Ht: *Homalictus tuiwawae*) where circle size indicates magnitude of sample size. f) Relationship between altitude and environmental temperature, g) relationship between altitude and precipitation, h) relationship between altitude and vapour pressure deficit (VPD). Filled circles indicate collection sites on the wet side of Viti Levu and open circles indicate sample sites on the dry side of Viti Levu.

To examine the climatic drivers of variation and co-variation in metabolic rate and respiratory patterns of bees, we collected individuals of *H. fijiensis*, *H. groomi,* Dorey et al., (2019), *H. tuiwawae,* Dorey et al., (2019), and *B. puangensis* from multiple sites on either side of the rain shadow along the altitudinal gradient of Viti Levu (Fig. S1e). We measured the metabolic rates of 203 individual bees at 25°C as rates of carbon dioxide production using flow-through respirometry. Of those bees that displayed discontinuous gas exchange, which was all but four individuals, we calculated their ventilation rate. We examined the relationships between the metabolic rate and

ventilation rate of bees and the climatic variables at their collection site using a strong inference framework whereby a set of models were selected *a priori* based on the explicit predictions of the MCA and hygric hypotheses (see Table 1 & Supplementary Box 1 for list of models tested). We accepted support for the MCA hypothesis if bees from cooler climates had higher metabolic rates and the only climate variable in the best model was temperature (Figure 2A). We accepted support for the hygric hypothesis if bees from drier climates had lower ventilation rates and the only climate variable in the best model was either vapour pressure deficit (VPD) or precipitation (Figure 2B), both of which are assumed to be indicative of aridity. In addition to testing the predictions of the MCA and hygric hypothesis, we also tested the hypothesis that ventilation rate and metabolic rate will be positively correlated, but that the strength of the relationship between these two traits will vary depending on the aridity of the environment (Figure 2C). All else being equal, insects with higher metabolic rates breathe more frequently [12–14], but we expect that in drier environments there will be stronger selection for insects with high metabolic rates to maintain lower ventilation rates to minimise water loss, which will result in a shallower relationship between ventilation rate and metabolic rate. In wetter environments where the risk of desiccation is lower, we expect that selection on low ventilation rate will be relaxed resulting in a steeper relationship between ventilation rate and metabolic rate.

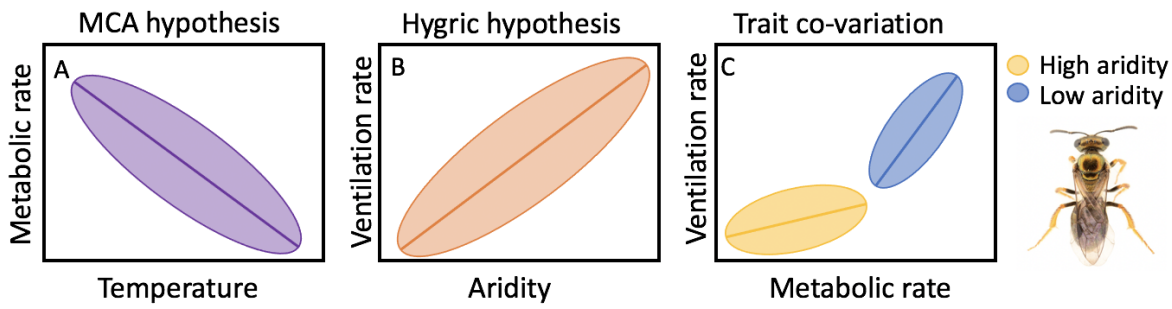

Figure 2. A) Conceptual illustration of the relationship between metabolic rate and temperature along an altitudinal gradient if the metabolic cold adaptation (MCA) hypothesis is supported. B) Relationship between ventilation rate and aridity (precipitation or inverse vapour pressure deficit (VPD)) if the hygric hypothesis is supported. C) Change in relationship between metabolic rate and ventilation rate depending on ardity (precipitation or VPD). Where there is reduced selective pressure to avoid desiccation, we expect that selective pressure on metabolic rate and ventilation rate will be relaxed, resulting in a steep relationship between metabolic rate and ventilation rate in areas of high precipitation or low VPD. *Homalictus fijiensis* photograph by James B. Dorey.

Methods

Animal collection

Bees were collected over 10 days in September 2019 on Viti Levu, Fiji. Viti Levu is divided by a mountain range that runs north to south, which creates a rain shadow on the western side of the island that receives significantly less rainfall than the eastern side (Fig. S1C). We collected bees from 16 sites along an altitudinal gradient of 6–1072 m above sea level (a.s.l.), with 10 of these sites being on the dry western side of the island and the other 6 sites being on the wet eastern side. Sites were sampled in a haphazard order over the 10 days and bees were collected by sweep-netting vegetation along road verges or in native vegetation. Individuals of *Braunsapis puangensis* were also collected directly from stem nests. The males and females of each species collected are known to be active year-round and are multivoltine (as per observation of M.P. Schwarz on multiple previous research trips to Fiji). Immediately upon capture, bees were placed in individual vials and stored in an insulated container. Inside the vials, bees were provided with a small piece of paper towel dipped in a 20% sucrose solution. On the day of collection, bees were transported to the laboratory to be measured that same evening.

Climatic variables

For each collection site, we downloaded historical climate data (1970-2000) at a 30-arc-second resolution from WorldClim2 [35] using the R package *raster* [36]. Since the MCA and hygric hypotheses make explicit predictions about how cool and dry climates shape metabolic rates and ventilation rates, respectively, we characterised the climates at each collection site using the variables of mean temperature (°C) of the coolest month (T_E) (July), mean precipitation (mm) of the driest month (July), and mean vapour pressure (VP, kPa) of the driest month (July) (Figure 1). VP was used to calculate the vapour pressure deficit (VPD, kPa) (drying power of the air) for bees during the driest month assuming that their respiratory surfaces are saturated with

water vapour (saturated water vapour pressure, SVP, kPa) and that their body temperature matches that of the environment (T_E, mean temperature of the driest and coolest month, °C) using the following equations:

$$SVP = 0.611exp\left(\frac{17.27 \times Te}{Te + 237.3}\right), \text{[37]} \quad (1)$$

$$VPD = SVP - VP \quad (2)$$

The range of environmental variables that each species was collected from is shown in Supplementary Table 1.

Trait measurements

We measured the metabolic rate and ventilation rate of individual bees by measuring their rates of carbon dioxide production (VCO_2, µl h^{-1}) at 25°C using a seven-channel flow-through respirometry system. The respirometry system was supplied with room air that was pushed through columns of soda lime and Drierite® to remove carbon dioxide and water vapour, respectively. Flow rates through each of the seven channels was regulated nominally to 100 mL min^{-1} by a mass flow controller (Aalborg, Model GFC17, Orangeburg, NY, USA). The volumetric flow rates produced by the flow controllers was measured using a Gilian Gilibrator-2 NIOSH Primary Standard Air Flow Calibrator with a low-flow cell (Sensidyne, LP, St Petersburg, FL, USA) and corrected to standard temperature pressure, STP (101.3 kPa and 0°C). To prevent bees from desiccating during measurements, the air was re-humidified after the flow controllers by passing the air through a humidifying chamber (a syringe of wetted cotton). The humidified air then flowed through a respirometry chamber (2.5 ml plastic syringe) containing an individual bee. Respirometry chambers were situated inside a temperature-controlled cabinet that maintained air temperature to 25 ± 1°C and kept bees in the dark. The excurrent air from the respirometry chamber then flowed through one of seven infrared CO_2/H_2O gas analysers (LI-COR, Model LI-840A, Lincoln, Nebraska, USA) that were calibrated with precision span gases (5.0 and 30.4 ppm

CO_2, Alphagaz – Air Liquide, Victoria, Australia) and measured CO_2 concentrations at a sampling rate of 1 Hz.

The fractional CO_2 concentration of the excurrent air (F_{ECO2}) from each respirometry chamber was recorded for 50 min. The fractional CO_2 concentration of the incurrent air when it was stable was measured for 2 min before and after each 50-min measurement period with an empty chamber, and a linear model was fitted to these data to estimate the CO_2 concentration of the incurrent air during the 50-min measurement period (F_{iCO2}). The rate of carbon dioxide production (VCO_2, µl h^{-1}) was then calculated using equation 3 (Lighton 2018) where FR is the flow rate (µl h^{-1}) corrected to STP (101.3 kPa and 0°C) accounting for water vapour dilution:

$$V_{CO2} = FR(F_{ECO2} - F_{iCO_2}) \quad (3)$$

The first 15 min of the 50-min measurement period was considered a settling period and was discarded from analysis. The lowest averaged VCO_2 over 20 min during the final 35 min of the measurement period was taken as the measure of metabolic rate for each individual. Bees that breathed discontinuously produced traces of carbon dioxide production that showed periods of minimal gas exchange interspersed by peaks in carbon dioxide production (Fig. S2). The number of peaks of carbon dioxide production observed during the 20-min period was used to calculate the ventilation rate (h^{-1}) of the bee (Supplementary Figure 1). In all, 96% of bees exhibited discontinuous gas exchange patterns. Those that had ventilations rates greater than 120 h^{-1} (n = 8) were removed from the analysis as it is likely they were stressed [24].

Immediately following metabolic rate measurements, bees were preserved in 100% ethanol to be sexed and weighed at a later date. Bees were sexed by visual assessment by examining the number of antennal segments (males have 13 and females have 12). Prior to weighing, the right hind leg of each individual was detached for species identification (see below). Dry mass was measured two months after metabolic rate measurements with specimens dried at 60°C for 48 h prior to being weighed (XP2U Ultra Micro Balance, Mettler Toledo, Victoria, Australia).

Bees were measured during the evening on the day that bees were collected from the field. Due to the logistical challenges of bee collection on Viti Levu, bees could not be collected from all sites each day. Consequently, on some nights, bees from multiple sites could be measured, but on other nights only bees from one site could be measured. Since the species and sex of the bees measured each night were mostly unknown (see below), measurements were conducted blind to the species and sex of bees in most cases (*B. puangensis* is easily differentiated from *Homalictus* species). After species were identified (see below), only those species with sample sizes greater than 10 were retained for analysis. In total, we measured 212 individuals of four species: *H. fijiensis* (n = 124), *H. tuiwawae* (n = 21), *H. groomi* (n = 16), and *B. puangensis* (n = 42).

Species identification

Homalictus species living at high altitudes are cryptic and, in many cases, can only be identified via male genitalia morphology, or via genetic comparison. To identify our *Homalictus* species collected from altitudes above 300 m a.s.l., we sent tissue samples (the right hind leg) to the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Ontario, Canada, to have the mtDNA COI gene fragment sequenced. A total of 84 samples were DNA barcoded using single-molecule real-time sequencing (SMRT49) using the PacBio Sequel platform (Pacific Biosciences, Menlo Park, CA, USA). Sequences were aligned to existing COI sequences taken from species identified by [33,38]. The invasive bee species, *B. puangensis*, which is generally restricted to the lowlands, is similar in size to *H. fijiensis*, but is distinguishable by its black cuticle and white facial marking [30,32].

Statistical analyses

We examined variation and co-variation in metabolic rate and ventilation rate within *H. fijiensis* and among species across climatic space using linear mixed models using the nlme package [39] in R Version 4.1.0 [40]. Using a strong inference approach, we tested suites of six models to test specific hypotheses that would support or reject the

MCA and hygric hypotheses, and to test whether the relationship between ventilation rate and metabolic rate changes across the aridity gradient (Table 1; see rationale behind hypothesis tests in Supplementary Box 1). The sixth model in each set was a null model that included altitude and no other environmental variables (Table 1). All models included $\log_{10}$ transformed dry body mass, sex and altitude as predictor variables, as well as the hypothesis of interest variable(s). Metabolic rate was also $\log_{10}$ transformed to ensure linear mixed effect model assumptions were met. We did not include VPD and temperature in the same models as VPD is strongly correlated with temperature. Models testing the hygric hypothesis included $\log_{10}$ transformed metabolic rate as a predictor variable to account for the effect of metabolic rate on ventilation rate as they are positively correlated. Multispecies models testing the MCA, hygric hypothesis and the way in which metabolic rate and ventilation rate covary included species as a factor within the models. Measurement block and channel were included as random factors within each model.

We compared the Akaike information criterion (AIC) of each model to determine which hypothesis best explained the variation in metabolic rate, ventilation rate and how traits covary across abiotic variables as per [18,41] using the MuMIn package [42]. We then evaluated the direction of the relationships between the traits of interest and abiotic variables within the best fitting models to make inferences on whether metabolic rate and ventilation rate are best explained by the MCA and hygric hypotheses, respectively, and if the relationship between metabolic rate and ventilation rate remains consistent across abiotic variables or if it becomes uncoupled. All models included $\log_{10}$ transformed body mass, sex and altitude as predictor variables, and an interaction between $\log_{10}$ transformed body mass and species were included within the multispecies models. The multispecies models testing the hygric hypothesis also included an interaction between $\log_{10}$ transformed metabolic rate and species. Only species that had sample sizes over 10 individuals were included in the multispecies analysis (four species). The packages ggplot2 [43] and cowplot [44] were used to produce data figures throughout the results.

350 **Table 1.** Hypotheses that were compared to test the metabolic cold adaptation
351 hypothesis, the hygric hypothesis, and examine if the way in which metabolic rate and
352 ventilation rate covaries changes with aridity. **-** indicates a negative relationship for
353 the hypothesis to be supported. **+** indicates a positive relationship for hypothesis to be
354 supported. o indicates we expect no relationship between the response variable and
355 the predictor variable.

Hypothesis predictor variable	**Metabolic rate (MR)** (testing MCA hypothesis)	**Ventilation rate (VR)** (testing hygric hypothesis)	**VR ~ MR * predictor variable * species** (Trait covariation depends on aridity)
T_{MIN} (mean temperature of coldest month, July)	-	o	o
P_{DRY} (precipitation of the driest month, July)	o	+	VR ~ MR relationship is steepest in low P_{DRY} environments
VPD (vapour Pressure deficit of the driest month, July)	o	-	VR ~ MR relationship steepest low VPD locations.
T_{MIN} + P_{DRY}	If T_{MIN} has a negative effect on MR, an MCA like pattern arises.	If P_{DRY} has a positive effect on VR, a hygric like pattern arises.	NA
T_{MIN} * P_{DRY}	If MR is highest at the lowest T_{MIN} * P_{DRY} combination an MCA like pattern will arise.	If VR is highest at the highest P_{DRY} * T_{MIN} combination, a hygric like pattern will arise.	VR ~ MR relationship steepest in cool wet locations.
Altitude (null hypothesis)	o	o	o

356

357

358 **Results**

359

360 *Testing the MCA within and among species*

361 The hypothesis that best explained variation in metabolic rate within *H. fijiensis* and
362 across species included average temperature of the coldest month (*H. fijiensis*:
363 estimate = -0.049 ± SE = 0.024, df = 89, t = -2.04, P = 0.045) (among species:
364 (estimate = -0.065 ± SE = 0.026, df = 160, t = -2.54, P = 0.012), and average
365 precipitation of the driest month as the test variables (*H. fijiensis*: estimate = -0.002 ±
366 SE = 0.0004, df = 89, t = -3.71, P < 0.005) (among species: estimate = -0.002 ± SE =
367 0.0004, df = 160, t = -4.53, P < 0.005) (Table 2 and see full model summary in
368 Supplementary Table 2 & 3). Metabolic rate was negatively correlated with both

temperature and precipitation, therefore an MCA like pattern is observed (Figure 3A & 3C), but the MCA hypothesis is not supported in its purest form. Within the *H. fijiensis* model comparison, two other models had ΔAIC values < 2, indicating that these models had similar model fit to the best fitting model (Table 2). However, two of the top three models included average temperature of the coldest month and all three included precipitation of the driest month, therefore, it is likely that both temperature and precipitation explain variation in metabolic rate in *H. fijiensis*, as explained by the additive top model.

Table 2. Testing the MCA within *H. fijiensis* (A) and among species (B). Model comparison table. Best fitting model with lowest AIC value is in bold. T_e = environmental temperature (°C), P_{dry}= mean precipitation of the driest month (mm), VPD = vapour pressure deficit (kPa). All models also included log_{10} transformed body mass, sex and altitude as predictor variables. *wi* indicates AIC model weight.

A) *Homalictus fijiensis* metabolic rate model comparison					
Model environmental factors	df	AIC	AICc	ΔAICc	*wi*
T_e + P_{dry}	**9**	**-128.3279**	**-126.7**	**0**	**0.53**
P_{dry}	10	-126.8611	-125.1	1.62	0.24
T_e x P_{dry}	8	-126.3840	-124.9	1.83	0.21
Altitude	7	-119.1432	-118.2	8.57	0.007
VPD	8	-117.9569	-116.7	10.04	0.004
T_e	8	-117.2654	-116.0	10.74	0.002
B) Multispecies metabolic rate model comparison					
Model environmental factors	df	AIC	AICc	ΔAICc	*wi*
T_e + P_{dry}	**12**	**-167.6970**	**-166.1**	**0**	**0.69**
T_e x P_{dry}	13	-165.7632	-163.8	2.22	0.23
P_{dry}	11	-163.1104	-161.7	4.33	0.08
Altitude	10	-153.1987	-152.1	14.00	0.001
VPD	11	-151.5211	-150.1	15.92	0.00
T_e	11	-151.2203	-149.8	16.22	0.00

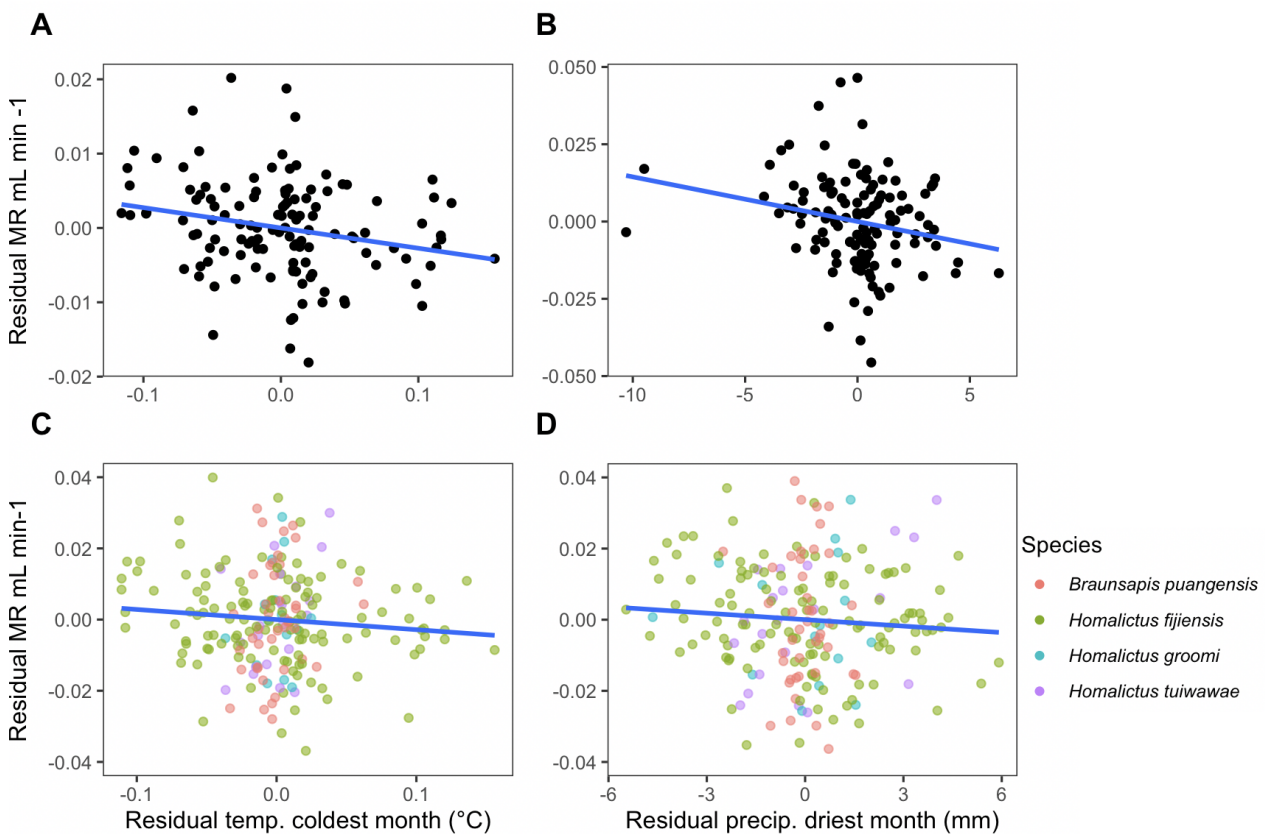

Figure 3. A) *Homalictus fijiensis* residual metabolic rate (mL min^{-1}) across residual average temperature of the coldest month, and B) residual precipitation of the driest month (mm). C) Residual metabolic rate of multiple Fijian bee species across residual temperature of the coldest month, and D) residual precipitation of the driest month.

Testing the hygric hypothesis within and across species

The model that best explained variation in ventilation rate within *H. fijiensis and* across species included precipitation of the driest month (*H. fijiensis*: estimate = 0.0005 ± SE = 0.0001, df = 89, t = 3.3, P < 0.005) (across species: estimate = 0.0004 ± SE = 0.00017, df = 160, t = 2.55, P = 0.012) (Table 3; see Supplementary Table 4 & 5 for full model coefficient summaries). Where ventilation rate increased with precipitation, supporting the hygric hypothesis (Figure 4).

Table 3. Testing the hygric hypothesis within *Homalictus fijiensis* (A) and across Fijian bee species (B). Best fitting model with lowest AIC value is in bold. T_e = environmental temperature (°C), P_{dry}= mean precipitation of the driest month (mm), VPD = vapour pressure deficit (kPa). All models also included log_{10} transformed body mass, sex, altitude and log_{10} transformed metabolic rate as predictor variables.

A) *Homalictus fijiensis* ventilation rate model comparison					
Model environmental factors	df	AIC	AICc	ΔAICc	*wi*
P_{dry}	**9**	**-345.05**	**-351.8**	**0.000**	**0.68**
T_e + P_{dry}	9	-353.43	-349.6	2.22	0.22
T_e x P_{dry}	9	-344.03	-347.3	4.55	0.07
Altitude	10	-351.57	-343.9	7.96	0.013
T_e	11	-349.66	-343.5	8.37	0.010
VPD	8	-345.14	-342.5	9.40	0.006
B) Multispecies ventilation rate model comparison					
Model environmental factors	df	AIC	AICc	ΔAICc	*wi*
P_{dry}	**12**	**-493.97**	**-492.3**	**0.00**	**0.56**
T_e + P_{dry}	13	-491.99	-490.1	2.26	0.18
T_e	12	-490.23	-488.6	3.47	0.08
Altitude	14	-489.99	-488.0	4.35	0.06
T_e x P_{dry}	12	-489.36	-487.8	4.57	0.057
VPD	11	-489.26	-487.6	4.71	0.053

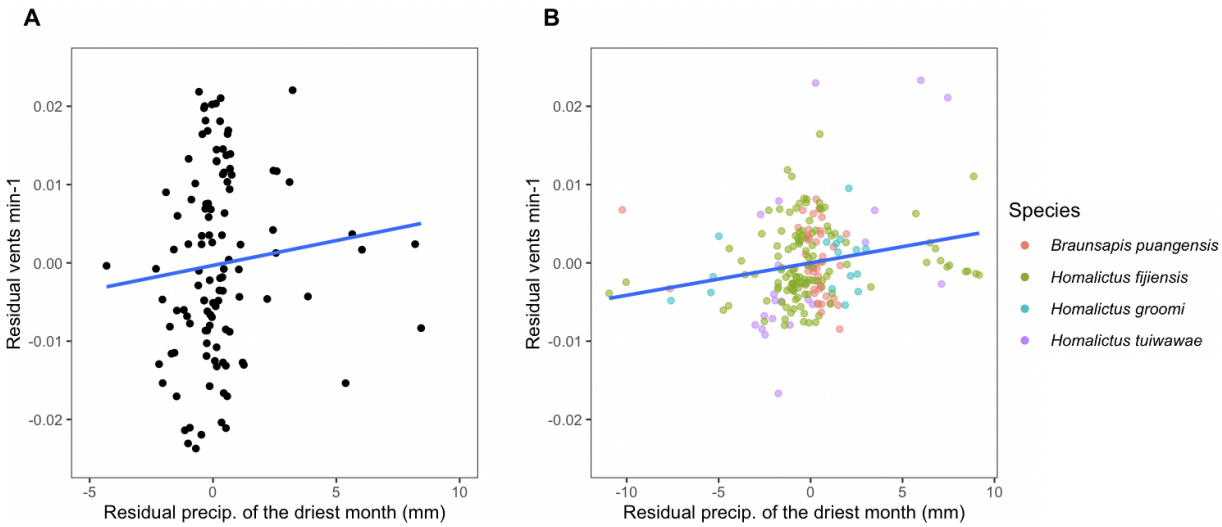

Figure 4. A) *Homalictus fijiensis* ventilation rate (ventilations min^{-1}) model residuals across precipitation of the driest month model residuals (mm) (for data visualisation two outlier points were removed with residual precipitation values less than -10, however, these individuals can be observed in panel B). B) Multispecies metabolic rate model residuals across precipitation of the driest month (mm) model residuals (*H. fijiensis* residual ventilation rate differs slightly in panels A and B due to different residuals being generated from different model structures).

Does the relationship between metabolic rate and ventilation rate co-vary with abiotic variables?

The model that best explained the the way in which ventilation rate covaries with metabolic rate across climatic variables within *H. fijiensis* and across bee species included an interaction between $\log_{10}$ transformed metabolic rate and precipitation of the driest month as the test variables (*H. fijiensis*: estimate = -0.0004 ± SE = 0.0005, df = 88, t = -0.81, P = 0.41) (among species: estimate = -0.0009 ± SE = 0.0005, df = 159, t = -1.68, P = 0.096) (see Supplementary Tables 6 & 7 for full model coefficient summaries). Despite the non-significant interaction term between metabolic rate and precipitation of the driest month in both the within *H. fijiensis* and across bee species models, these models had notably lower AICc values than all other test hypotheses (Table 4). The negative model estimate values stated above indicate that bees that inhabit drier environments have higher ventilation rates per mL of CO_2 exchanged per

minute than bees that inhabit wetter environments within species and among species (Figure 5).

Table 4. A) *Homalictus fijiensis* trait covariance model comparison table. B) Multispecies trait covariation model comparison. Best fitting model with lowest AICc (sample size corrected AIC) value is in bold. T_e = environmental temperature (°C), P_{dry}= mean precipitation of the driest month (mm), VPD = vapour pressure deficit (kPa).

A) *Homalictus fijiensis* trait covariance model comparison					
Testing how trait covariance changes with climatic variables	df	AIC	AICc	ΔAICc	*wi*
MR x P_{dry}	**10**	**-352.12**	**-350.2**	**0**	**0.93**
Altitude	8	-345.14	-343.9	-6.29	0.04
MR x T_e	10	-343.21	-341.3	-8.91	0.01
MR x T_e x P_{dry}	14	-344.36	-340.5	-9.67	0.007
MR x VPD	10	-342.34	-340.4	-9.78	0.007
B) Multispecies trait covariance model comparison					
Testing how trait covariance changes with climatic variables	df	AIC	AICc	ΔAICc	*wi*
MR x P_{dry}	**13**	**-507.33**	**-505.4**	**0.00**	**0.94**
MR x T_e x P_{dry}	17	-501.45	-498.1	7.26	0.025
Altitude	12	-498.41	-496.8	8.63	0.013
MR x T_e	13	-498.34	-496.4	8.99	0.011
MR x VPD	13	-497.19	-495.3	10.14	0.006

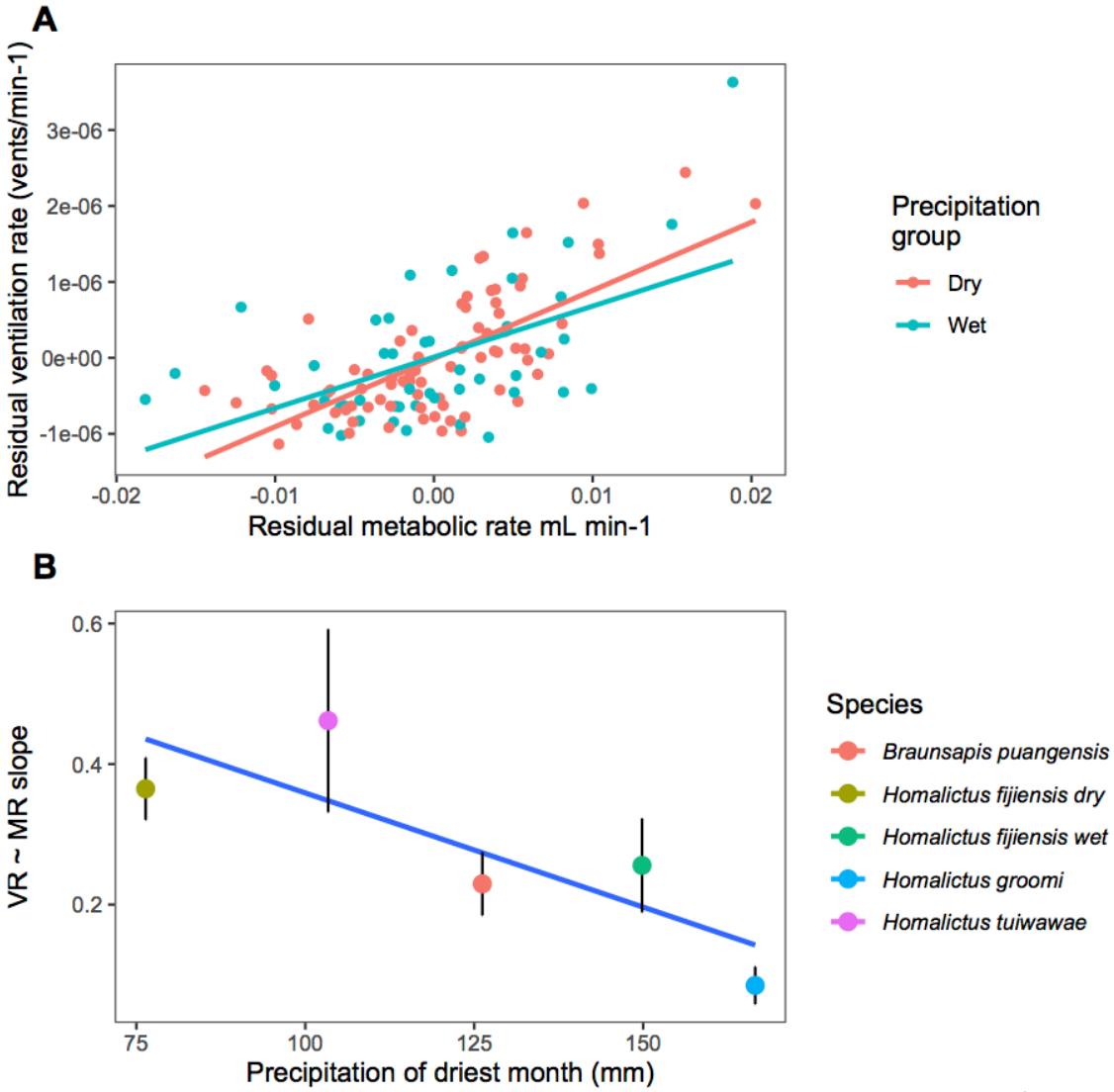

Figure 5. A) *Homalictus fijiensis* residual ventilation rate (ventilations min^{-1}) by residual metabolic rate (mL min^{-1}). B) Multispecies relationship between ventilation rate and metabolic across precipitation of the driest month. To visually show how the relationship between metabolic rate and ventilation rate changes depending on precipitation of the driest month we grouped *H. fijiensis* into precipitation groups, 'dry = 57 - 124 mm' or 'wet = 125 - 190 mm'. This is for visual representation only, and the parameter estimates from the model should be used to gain a true understanding of how the relationship between metabolic rate and ventilation rate changes across the precipitation of the driest month gradient.

Discussion

Adaptive hypotheses that explain variation in physiological traits along climatic gradients make explicit predictions based on the expected direct effects of abiotic variables on physiological function. The metabolic cold adaptation (MCA) and hygric

hypotheses, for example, predict that metabolic rates and gas exchange patterns evolve in response to the effects of temperature and water availability on these two traits, respectively [6,10]. Whether these hypotheses are supported within and among species and across environments has been the centre of debate over the last few decades [6,7,10,19,45]. However, despite metabolic rate and gas exchange patterns being inherently linked, how they covary across climatic conditions is rarely considered. Understanding how climate shapes trait relationships is likely to be critical for improving our understanding of the mechanisms that underpin species distributions and survival with changing environments [46,47]. We discuss how metabolic rate and ventilation rate change across climatic gradients independently and then simultaneously to explore how these traits might evolve together.

We found that the MCA hypothesis was not supported in its purest form in Fijian bees. Although we found a negative relationship between metabolic rate and temperature, as predicted by the MCA hypothesis, the best model that explained variation in metabolic rate also included precipitation. We expected that if precipitation did explain variation in metabolic rate, that metabolic rate would be lowest in dry environments and highest in wet environments as a mechanism to avoid desiccation (Supplementary Box 1). However, we found that metabolic rate was negatively correlated with precipitation (Figure 3B & 3D). This unexpected pattern might be explained by variation in environmental productivity. Productivity is usually greatest in regions of high precipitation, and biodiversity is often positively correlated with productivity [48]. In Fiji, precipitation increases with altitude and so does native bee species diversity, where only one endemic species, *H. fijiensis,* is common in the lowlands and ~ 22 other endemic *Homalictus* species are found in the highlands (> 800 m a.s.l.) [33,38]. Land surface area decreases with altitude in Fiji, leading to an environment that is small, but productive, and contains high endemic bee diversity. Therefore, potentially high competition for resources between species has led to an environment where a lack of energetic resources could be reducing metabolic rates in bees that inhabit high precipitation high altitude regions. Low metabolic rates might be favoured in environments with high competition or fewer resources following the ‘compensation’ hypothesis which suggests that individuals with low resting metabolic rates will have higher fitness because they have lower self maintenance costs and can devote more energy towards growth and reproduction [49]. However, this hypothesis contradicts

findings by [50] which shows that individuals with higher metabolic rates have greater competitive ability.

Variation in ventilation rate within *H. fijiensis* and across Fijian bee species was also best explained by precipitation of the driest month (Table 3 & Figure 4). Ventilation rate increased with precipitation, supporting the idea that ventilation rate will be suppressed in dry environments to avoid desiccating (hygric hypothesis), but selective pressure on ventilation rate will be relaxed in wet environments. Generally, the hygric hypothesis is tested by examining the type of breathing pattern that insects employ across different environments (e.g. continuous, cyclic or discontinuous), but here we show that species that exhibit discontinuous gas exchange can further regulate the frequency at which they exchange gas, likely in response to aridity. However, it is interesting to note that a model that included vapour pressure deficit (drying power of the air), the other aridity metric, offered the least amount of support in explaining variation in metabolic rate and ventilation rate within *H. fijiensis* and across bee species. Because of the rain shadow in Fiji, vapour pressure deficit and precipitation are only weakly correlated, as can be observed in Figure 1. Thus, bees that inhabit regions that experience more rainfall might have greater access to water in the environment, therefore reducing desiccation stress, even if the drying power of the air remains high.

When we examined how metabolic rate and ventilation rate covary across climatic space, we found that within *H. fijiensis* and across species, those that inhabit the driest environments have higher ventilation rates per unit CO_2 expelled (steeper ventilation rate ~ metabolic rate slopes) (Figure 5). Therefore, bees that inhabit drier regions have high ventilation rates but only exchange a small volume of CO_2 per ventilation, and that bees in wet environments have low ventilation rates, but each ventilation exchanges a greater volume of gas. Therefore, we suspect that bees in dry environments are using shallow ventilations as a mechanism to breathe while limiting the amount of water that can leave their system and reduce the volume of air that can diffuse through their tracheole system and desiccate them. Previous studies have found that some insects fill their tracheoles with water to reduce the surface area of their respirometry surfaces, potentially as a mechanism to avoid desiccating [51]. Because we re-humidified our respirometry system to avoid desiccating the bees, we

were unable to measure rates of water loss while measuring metabolic rate, which would have been useful in determining if bees with high frequency small volume ventilation rates lose less water than bees with slower frequency high volume ventilations. Future studies should seek to examine how rates of water loss correlate with metabolic rate-ventilation rate patterns in the future.

As we found that the correlation between metabolic rate and ventilation rate changed across the precipitation gradient, it suggests that while ventilation rate and metabolic rate are linked (they are positively correlated across all species) we could be observing a physiological trait trade-off. While we cannot determine whether the change in correlation between traits across the precipitation gradient is due to correlational selection or genetic correlation, the fact that species from different environments have different trait relationships suggests that there is scope for metabolic rate and ventilation rate to evolve independently of each other in response to variation in climate. However, while the two traits might be evolving independently of each other, they remain correlated, and the extent to which each trait can evolve independently in response to variation in climate remains an exciting research question for the future.

We believe it is critical to examine organisms as a mosaic of traits to improve our understanding of organismal responses to changing environments [47,52,53]. Yet traits are still commonly measured and considered in isolation with very few examples of how traits coevolve across environmental space, especially in wild populations [but see 47,54]. Our study shows that the correlation between metabolic rate and ventilation rate changes across a precipitation gradient, and that these traits could be evolving independently of each other in response to abiotic factors. Further work is needed to determine whether the observed phenotypic correlation is underpinned by the same or different alleles and to examine evolutionary responses of linked traits that are important for species survival with climate change. Variation in the strength of trait relationships could have implications for the way in which species evolve with further anthropogenic climate change [55]. As organisms are composed of multiple interacting traits and adaptation is a multidimensional problem, we believe it is critical for research to explore how climate tolerance traits are linked and evolve across landscapes together as we move further into the Anthropocene.

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