The respiratory metabolism of overwintering paper wasp gynes (Polistes dominula and Polistes gallicus)

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Abstract. Winter in climatic regions with low temperatures is a challenge for overwintering insects. They are exposed to temperature extremes, which directly cause mortality or lead to energy depletion. The winter hibernaculum of paper wasp gynes protects from predators and rain, but only poorly from ambient temperature. In order to detect physiological adaptations to differing climates, we compared the respiratory metabolism of overwintering gynes of two polistine species from the Mediterranean climate in Italy (Polistes dominula and Polistes gallicus), and of one species from the temperate climate in Austria (Polistes dominula). The wasps’ CO₂ emission was measured with stop-flow respirometry in a temperature range from 2.5 to 20 °C. The mass-specific standard (resting) metabolic rate, the main type of metabolism of the dormant insects during overwintering, increased exponentially with ambient temperature but was suppressed in comparison to individuals measured in the summer, which conserves the energy stores. In addition, it was lower in the Mediterranean species (P. dominula and P. gallicus) in comparison to the temperate species (P. dominula), especially at higher temperatures. The active metabolic rate was suppressed to a similar amount. The suppressed metabolism of the overwintering Mediterranean gynes could be an adaptation to the higher winter temperatures to prevent a premature depletion of the energy resources.

Key words. Gynes, Polistes dominula, Polistes gallicus, respiratory metabolism, temperature.

Introduction

Winter in climatic regions with low temperatures is a challenge for overwintering insects. Many insects hibernate at sites that provide only poor protection from the harsh conditions of winter. They are exposed to temperature extremes, which directly cause mortality or lead to water deficit and energy depletion, threatening their survival. Most of these overwintering insects spend the winter in a state of dormancy (diapause or quiescence; Koštál, 2006), in which activity is low and feeding ceases, and the metabolic rate is suppressed (Hahn & Denlinger, 2011). Most of them are ectothermic, and as such, their body temperatures are generally similar to the ambient (microclimate) temperature. As they generally do not feed, the energy stores with which they enter dormancy must fuel the mechanisms that protect against cold and desiccation, and provide the fuel for the basal metabolism throughout the winter. The energy reserves remaining at the end of the cold period are determined by the climate and the thermal conditions (microclimate) experienced during winter. Anthropogenic climate change is altering mean temperatures as well as winter temperatures (IPCC, 2014; Pachauri et al., 2015). The question arises, what warmer winter temperatures mean for overwintering insects. Is it beneficial or is it unfavourable for them? Metabolic rate is a function of temperature in ectothermic species (e.g. Gillooly et al., 2001). An acute increase in temperature will result in an approximately exponential increase in metabolism. Some studies showed that increased winter temperatures can decrease the fitness of dormant insects. Warmer overwintering temperatures lead to higher rates of resource consumption and a concurrent depletion of stored reserves, which negatively impact survival and fecundity as only reduced resources are available for reproduction in the spring (e.g. Bosch & Kemp, 2003; Irwin & Lee, 2003; Williams
The evolution of metabolic rate-temperature reaction norms is of fundamental importance to physiological ecology (Terblanche et al., 2009). Metabolic cold adaptation describes a macrophysiological pattern, whereby respiratory estimates of metabolic rates of ectotherms from cold environments, typically from high latitudes or altitudes, tend to be elevated relative to those from warm environments (e.g. Chown, 1997; Chown & Gaston, 1999; Addo-Bediako et al., 2002; Okawa et al., 2006; Terblanche et al., 2009; Bozinovic et al., 2011; Williams et al., 2016). Alternatively, it has been proposed that metabolic cold adaptation may manifest itself in the form of an altered sensitivity of the relationship between temperature and metabolic rate, mainly realized by a metabolic increase at low temperatures (Pörtner, 2002). A study of sub-arctic invertebrates has shown that northern species are more sensitive than southern species (Nielsen et al., 1999), which suggests that the relationship between thermal sensitivity and geographical latitude seems to be important. In polistine wasps, this would mean that wasps from temperate climate should have a higher metabolic rate and sensitivity than individuals from the Mediterranean climate. Therefore, we compared the respiratory metabolism of overwintering gynes of two species (Polistes dominula and Polistes gallicus) from differing climates. We hypothesized that populations would be locally adapted to their (overwintering) environments. We wanted to assess whether adaptations are associated with changes in metabolic rates. For this purpose, we measured the metabolic rate (CO₂ production) of resting and active individuals. In insects, the standard metabolic rate (SMR) is usually equated with resting metabolism. It represents the energetic costs of basic subsistence and determines an individual’s minimum energy requirement. It accounts for up to 50% of individual energy expenditure and is linked with fitness, showing correlations with traits such as growth and survival (for details see Burton et al., 2011). In the case of overwintering wasps, it has high importance as they spend most of the time dormant in their hibernaculum. Clarke (2004) suggested that an organism’s resting metabolic rate appears to be set by an evolutionary trade-off between costs, benefits and ecological lifestyle. The resting metabolic rate may thus be regarded as the energetic cost of evolutionary temperature adaptation. The resting metabolism in summer individuals (female workers) of polistine wasps increases exponentially with ambient temperature (Käfer et al., 2015; Kovac et al., 2017). It is not known whether the wasp gynes’ metabolism shows the same strong temperature dependence. The increasing winter temperatures due to climate change could seriously threaten wasps, as higher temperatures during overwintering would result in a higher metabolic rate, which could untimely reduce their energetic storage fuels before they can leave their winter quarter for foraging. To find out whether acclimation to winter conditions affects the wasps’ metabolic capacity, and thus their ability to move during warmer winter periods (e.g. Glazier, 2015a), we also measured their active metabolic rate.

Here, we compare the metabolic rate-temperature relationships for wasps from different climates to better understand the energetic demands of overwintering, and the potential physiological adaptations thereof. We also coupled our measurements of metabolic rate with representative microclimate temperature recordings from these locations to provide energy consumption estimates of overwintering. Together, these data allow insights into climate change vulnerability for these species based on resource depletion.

Materials and methods

Study species and origin of samples

The experiments were conducted with gynes (fertile females) of overwintering Polistes dominula and Polistes gallicus in the years 2017–2019. The individuals of a P. dominula population from Austria (P. dominula AT), a P. dominula population from Italy (P. dominula IT) and P. gallicus (P. gallicus IT), an Italian sister species of P. dominula, were collected from their winter hibernacula in November (Fig. 1; bird nesting boxes in Gschwendt and Fernitz, Styria, Austria; candle lanterns at a cemetery in Trespiano, Tuscany, Italy). After the transport to the laboratory (in Austria), the measurements started immediately and were conducted within 1 week after collection.

Experimental set-up and measurement procedure

Prior to the experiments, the wasps were weighed with an accuracy of 0.1 mg (Shimadzu AUW-120DV, Nishinokyo
Kuwahara-cho, Nakagyo-ku, Kyoto, Japan). Then the wasps were put in small plastic tubes (Fig. S2, length 35 mm, diameter 9 mm, volume 2.23 mL) which worked as measurement chambers. Eight of these measurement chambers were arranged in parallel and placed in a water bath (Julabo F33 HT, JULABO Labortechnik GmbH, Seelbach, Germany) with open lid, to allow observation and recording the wasps’ activity. The measurement chambers were submerged in water to guarantee a constant temperature of ±0.1 °C accuracy during the experiments. The experiments were conducted in a temperature range from 2.5 to 20.0 °C in 2.5 °C steps. Eight wasps were measured simultaneously on one day. At least 16 individuals of each species were tested at each experimental ambient temperature (T_a). The wasps were put in the measurement chambers and remained there for about 4 h at a certain test temperature. In two thirds of the experiments, the wasps were tested at only one temperature per day and on the next day the temperature was changed. In the remaining third, the test temperature was changed on the same day, and the experiment was continued for further 4 h. The experiments were conducted in the dark (realized during the daytime by darkening the laboratory). Measurements started not before half an hour of habituation time in the measurement chamber. The wasps’ behaviour during the experiments was recorded with near-infrared light by means of a night vision video camera (Sony GDR-CX730E, Sony Europe Limited, Vienna, Austria) installed above the water bath, for later evaluation of their activity. In the video analysis, the behaviour of the wasps was classified in two categories, ‘no activity’ (resting or standard metabolic rate, SMR) and ‘activity’ (mainly grooming or walking; active metabolic rate, AMR). Every action had to last for at least 5 s to be categorized as a separate behaviour event. To determine resting metabolism (SMR) the wasps had to be categorized as resting for the whole 7 min of one CO2 measurement interval. In further steps of the analysis, the metabolic rate of the active and resting wasps was calculated, by averaging the CO2 production rate of a certain period (active or resting). As the wasp species differed significantly in their weight, the mass-specific CO2 production rate (VCO₂, μL g⁻¹ min⁻¹) was calculated.

In the experiments described above, the wasps’ CO2 emission was measured, which is commonly used as an indirect measure of an organism’s metabolic rate. Eight respirometry measurement chambers were connected to an eight-channel multiplexer (RM Gas Flow Multiplexer, Sable Systems, Las Vegas, Nevada), working in a stop-flow measuring arrangement. The multiplexer controlled the sequential flushing and shut-off of the metabolic chambers, which allowed the simultaneous measurement of eight individuals by turns. During the flushing phase, the metabolic chambers were perfused with humidified air (50% rH) at a fixed flow rate of 144 mL min⁻¹. After the flushing phase, the metabolic chamber was closed. The duration of the flushing phase of one chamber was 1 min, therefore the closed phase was 7 min. The multiplexer was connected to a differential infrared carbon dioxide gas analyser (DIRGA; U拉斯 14, ABB, Züri, Switzerland) which measured the insects’ CO2 release with an accuracy of ~2 ppm. To maximize the system sensitivity (<0.2 ppm), the air was taken from outside the laboratory. Before it entered the reference tube of the DIRGA, the air was led through a 10 L container to dampen fluctuations in CO2 content, passed the pump and mass flow controllers (0–1000 mL min⁻¹, Brooks 5850 S), and then passed through another container (5 L) for additional CO2 and pressure fluctuation damping. To maintain a relative humidity of about 50% in the measurement chambers, the air was humidified by passing it through two bottles filled with distilled water (see Stabentheiner et al., 2012). The air was dried by Peltier-driven cool traps (10 °C) before it entered the URAS reference and measurement tubes (where it was heated to 60 °C). The volumes (nL) of CO2 production reported in this paper refer to standard (STPS) conditions (0 °C, 101.32 kPa = 760 Torr). The CO2 release was recorded at 1-second intervals. At the beginning and at the end of each experimental run, the gas analyser was calibrated automatically in zero and end point by the use of internal calibration cuvettes, and the data were corrected for any remaining drift or offset.

The SMR of the overwintering gynes was compared with the SMR of summer individuals (workers) (from Kovac et al., 2020) which had been measured with the same equipment and the same method in the same period (2017–2019).

In 2018, we conducted additional experiments to determine the respiratory quotient (RQ) with the same individuals who were present in the respiratory measurements. The RQ was determined for energetic calculations of the wasps’ energy
turnover during the winter. The experiments were performed at 5, 15 and 25 °C with seven individuals of each species (P. dominula from AT, P. dominula IT and P. gallicus IT). Seven wasps were placed in the same respiratory measurement chambers as described above and remained there from about half an hour to 5 hours, depending on the test temperature. In order to accumulate enough CO₂ and O₂-depleted air for an accurate measurement, experimental time was longer at low temperature than at high temperature. The respirometry measurement chambers were connected to the multiplexer. Commercial dried air was passed to the reference and measurement channels (in parallel mode) of a serial arrangement of the DIRGA and an Oxzilla 2 differential oxygen analyser (Sable Systems International, Las Vegas, Nevada). The multiplexer switched the measurement channels between the eight chambers in sequential order. After leaving the measurement chambers the air passed a desiccant (Drierite; W. A. Hammond Drierite Co. Ltd., Xenia, Ohio) before it entered the DIRGA and Oxzilla. The difference between measurement and reference channels was used to compensate for any instrument drift and offset during evaluation. Data acquisition was done with the DIRGA CO₂ gas analyser system software (Centrol 5, Harnisch, Austria). After drift and offset correction, the accumulated CO₂ and consumed O₂ was calculated by integrating the signals against time. Then, the respiratory quotient was calculated as the quotient of the integrals ($RQ = \frac{\int CO_2}{\int O_2}$). The system was calibrated at least once every day prior to the experimental runs.

**Microclimate measurement**

In order to measure representatives of the microclimate during overwintering, we installed MSR data loggers (MSR Electronics GmbH, Seuzach, Switzerland) at typical winter hibernacula, where we had observed overwintering of the wasps in several years. In Austria, we installed a logger in a bird-nesting box in Gschwendt (Styria, Austria; Fig. 1) beside a cluster of P. dominula. In Italy, we placed the logger in a candle switched at a cemetery in Trespiano (Tuscany, Italy; Fig. 1), where these shelters were used by mixed clusters of P. dominula and P. gallicus. The temperature was recorded from 9th of November 2018 until 2nd of April 2019 in 10-min intervals. During this time, the wasps remained in the hibernaculum. Mean, median, minimum and maximum temperatures of the air inside the hibernaculum were calculated using an MS-Excel implementation of the microclimate macros developed by Sinclair (2001). The number of events and time spent above 10 °C, and the number of events and time spent below 0 °C were determined for each hibernaculum to describe the microclimate (Terblanche et al., 2005). These temperatures were compared using Kruskal–Wallis rank analysis of variance (ANOVA) by Statgraphics software (Statgraphics Centurion XVI, StatPoint Technology Inc., The Plains, Virginia) followed by non-parametric multiple comparisons.

**Data analysis and statistics**

All calculations were performed with MS Excel (Microsoft Corporation, Redmond, Washington) and with Origin 2017 software (OriginLab, OriginLab Corporation, Northampton, Massachusetts). Curve fittings were performed with Origin software. The average values for the evaluated parameters mentioned in the results derive from the curve fittings. The accompanying statistics was done with Statgraphics Centurion XVI (StatPoint Technology Inc., The Plains, Virginia) and IBM SPSS Statistics (SPSS Inc., Chicago, Illinois). Curve fittings and statistics were conducted with single values of each individual and with means of each individual. We first examined with a factorial analysis of covariance (ANCOVA) whether standard (SMR) and active (AMR) metabolic rate depended on temperature and differed between species. We linearized the metabolic data by log-transformation. The model included $log_{10}$-transformed metabolic rate ($log_{10}$ MR in μL CO₂ min⁻¹) as dependent variable, species and test temperature as categorical and continuous factors and mass as covariate. As the species differed significantly in their mass, for a further pairwise comparison of the species we used an ANOVA with mass-specific SMR ($log_{10}$ MR in μL CO₂ g⁻¹ min⁻¹) as dependent variable, and ambient temperature and species as factors to determine differences in intercept and slope of regressions. ANOVA was conducted with all 7-min-interval values, which considers both intra- and inter-individual variation. In addition, ANOVA and ANCOVA with means of individuals, which revealed similar results, are presented in Tables S5 and S6.

We presumed that the wasps were mainly calm and immobile during overwintering. Therefore, we used the SMR data for energetic calculations. The wasps’ mean energy turnover (mW g⁻¹) was calculated with the values of the metabolic fit functions (Table S2) and the microclimate temperature recordings inside the hibernaculum (Fig. 3). For this purpose, we first transformed the CO₂ production to O₂ consumption with the respiratory quotient (RQ) determined for each species, and then multiplied the O₂ consumption with the adequate caloric (oxyjoule) equivalent (e.g. Erregger et al., 2017). Then the mean energetic turnover was calculated chronologically for the 10-minute intervals (derived from the temperature recordings in the hibernaculum). As our lowest test temperature was 2.5 °C and there were many temperature values below zero (resulting in negative metabolic values), we extended our fit curves with a linear interpolation to an estimated metabolic rate of 0.001 μL min⁻¹ g⁻¹ CO₂ at −5 °C, according to measurements of the metabolism of codling moth larvae (Thaumatotibia leucotreta) by Boardman et al. (2013). Below −5 °C metabolism was assumed to be zero (0). We performed the Kruskal–Wallis Rank test to compare the temperature recordings of the hibernaculum and the energetic calculations.

**Results**

**Standard metabolic rate (SMR), activity metabolic rate (AMR) and respiratory quotient (RQ)**

The mean weight of the investigated gynes was 128.6 ± 18.85 mg ($n$ = 18) for Polistes dominula AT, 111.7 ± 16.19 mg ($n$ = 16) for Polistes dominula IT and 75.8 ± 16.49 mg ($n$ = 22) for Polistes gallicus IT. The weights
differed significantly between the species and populations (Kruskal-Wallis test, $H = 35.3324$, d.f. = 2, $P < 0.0001$), and therefore we used the mass-specific metabolic rate for the further comparisons.

The standard and the active metabolic rate (SMR and AMR) of all wasps increased with ambient temperature in a typical exponential course (Fig. 2, Fig. S2; Tables S2, S3 and S5; ANCOVA, $P < 0.0001$). The lowest mean value of the mass-specific SMR ($\dot{VCO}_2$) was 0.05 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. dominula AT, 0.16 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. dominula IT, and 0.18 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. gallicus IT, determined at an experimental ambient temperature ($T_a$) of 2.5 $^\circ$C. The $\dot{VCO}_2$ increased to 1.08 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. dominula AT, 0.67 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. dominula IT and 0.94 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. gallicus IT at 10 $^\circ$C. At 20 $^\circ$C it reached 4.18 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. dominula AT, 3.68 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. dominula IT and 3.60 $\mu$L g$^{-1}$ min$^{-1}$ in $P$. gallicus IT, respectively (Fig. 2, Table S1). A comparison of the SMR between species revealed that they differed significantly (Fig. 2; ANCOVA, $F$-quotient = 101.0, $P < 0.0001$). The pairwise comparison of the SMR revealed that $P$. dominula AT differed significantly from the Italian populations (Table S6; $P$. dominula AT vs. $P$. dominula IT: ANOVA, $F$-quotient = 1151.16, $P < 0.0001$; $P$. dominula AT vs. $P$. gallicus IT: ANOVA, $F$-quotient = 1173.88, $P < 0.0001$).

Active wasps were mainly walking and grooming. Their AMR was significantly elevated above the SMR, and increased in an exponential course similar to the SMR. A comparison of the AMR between species revealed that they differed significantly (Fig. 2; ANCOVA, $F$-quotient = 13.51, $P < 0.0002$). The mean AMR was 1.3- to 6.5-fold higher than the mean SMR in $P$. dominula AT, 1.6- to 8.3-fold higher in $P$. dominula IT and 1.6- to 4.8-fold higher in $P$. gallicus IT, respectively.

The RQ was 0.78 ± 0.08 ($n = 7$) for $P$. dominula AT, 0.80 ± 0.10 ($n = 7$) for $P$. dominula IT, and 0.78 ± 0.09 ($n = 7$) for $P$. gallicus IT. Values did not differ significantly (Kruskal-Wallis test, $H = 1.6860$, d.f. = 2, $P = 0.4304$).

**Microclimate and energetics**

From November 2018 until April 2019, we measured a mean temperature of 4.4 ± 7.1 $^\circ$C (median = 3.2 $^\circ$C, min = −10.0, max = 33.0, range = 43.0; $n = 20668$) in the hibernaculum of $P$. dominula in Austria, and 9.9 ± 5.77 $^\circ$C (median = 8.9, min = −2.5, max = 33.7, range = 36.2; $n = 20668$) in the mixed hibernaculum of $P$. dominula and $P$. gallicus in Italy. The mean and median temperatures of the hibernacula differed highly significantly (Table S4; Fig. 3; Kruskal-Wallis test, $H = 10707.9$, d.f. = 1, $P < 0.0001$, $n = 20668$). The absolute maximum temperatures, as well as the number of events and time above 10 $^\circ$C was higher in the Italian hibernaculum. The absolute lowest temperature was measured in Austria, and the number of events and the time below 0 $^\circ$C was higher in Austria. The two hibernacula differed significantly in these parameters (for details see Table S4).

The mean energy turnover ± SD during the observation period was calculated with the 10-min values (based on SMR, RQ and hibernacula temperature) and then averaged to get daily means. Means of the daily averages were 0.042 ± 0.0433 mW
in *P. dominula* AT, $0.058 \pm 0.0576 \text{mW}$ in *P. dominula* IT and $0.040 \pm 0.0278 \text{mW}$ in *P. gallicus* IT. *P. dominula* IT differed from the other populations (Fisher’s LSD test, $P < 0.05$, $N = 144$ days). Means of the daily mass-specific averages of energy turnover were $196.2 \pm 201.50 \text{mW g}^{-1}$ in *P. dominula* AT, $316.2 \pm 314.33 \text{mW g}^{-1}$ in *P. dominula* IT and $329.7 \pm 227.13 \text{mW g}^{-1}$ in *P. gallicus* IT. *P. dominula* AT differed from the other populations (Fisher’s LSD test, $P < 0.05$, $N = 144$ days).

**Discussion**

In our study on overwintering gynes of paper wasps from Austria (*Polistes dominula* AT) and from Italy (*Polistes dominula* IT and *Polistes gallicus* IT), we could show a typical exponential temperature dependence of the standard (resting) respiratory metabolism (SMR) (Fig. 2). This exponential increase is similar to that observed in summer individuals (females) of polistine wasps (Käfer et al., 2015; Kovac et al., 2017, 2020). The metabolic performance (level), however, differed strongly. Overwintering gynes of all populations acclimated to winter conditions by reducing their metabolism by a factor of 0.51–0.81 in comparison to summer individuals measured with the same setup by Kovac et al. (2020) (Table 1). Even their active metabolic rate did not exceed the resting metabolic rate of summer individuals (workers). This acclimation to winter conditions is an important measure to save energy, in addition to the anyway present acute effect of low temperature on metabolism (Hahn & Denlinger, 2011).

The temperature dependence of the metabolic rate is responsive to environmental variation, and the metabolic rate response to temperature can differ among populations and among species (Somme & Block, 1991; Hoffmann, 1995; Addo-Bediako et al., 2002; Chown & Nicolson, 2004). In our study, we could show that even populations of the same species (*P. dominula*) as well as of a closely related sister species (*P. gallicus*) differ in their metabolic response (sensitivity) to temperature. The species originating from the cooler temperate Austria (*P. dominula* AT) exhibited a higher mass-specific standard metabolic rate than the wasps from the warmer Mediterranean Italy (*P. dominula* IT, *P. gallicus* IT). This was especially pronounced at higher experimental temperatures ($T_a \geq 10^\circ \text{C}$, Fig. 2). We suggest that, in addition to winter acclimation, their metabolism adapted to the different microclimate conditions in the evolutionary process. At first sight, a comparison of summer individuals of *P. dominula* from the temperate climate in Austria and *P. gallicus* from the Mediterranean climate in Italy (Kovac et al., 2020), where no difference in the species’ SMR had been detected, seems to contradict this interpretation. This similarity in summer wasps, however, is explained by the sheltered nesting of *P. dominula* in the temperate region, which provides them with microclimatological conditions similar to the Mediterranean climate. The differences we found in overwintering gynes are in accordance with a study of Williams et al. (2015a) on fall webworms (*Hyphantria cunea*; Lepidoptera: Arctiidae). Northern ecotypes of these insects had a lower thermal sensitivity of metabolism, leading to higher metabolic rates at cool temperatures. Pupae overwintering at warm, energetically demanding southern temperatures suppressed their metabolic rates in autumn. These findings are in agreement with the metabolic cold adaption hypothesis (e.g. Clarke, 1991; Chown et al., 1997; Chown & Gaston, 1999; Addo-Bediako et al., 2002; Oikawa et al., 2006; Terblanche et al., 2009; Bozinovic et al., 2011; Williams et al., 2016). This hypothesis states that selection for colder environments may drive a higher respiratory metabolism and thus contribute to the pattern of metabolic cold adaptation in terrestrial arthropods. Though many studies have found intraspecific and interspecific variation in metabolic rate-temperature relationships associated with living in cold or warm habitats (Chappell, 1983; Terblanche et al., 2009), this hypothesis is quite contradictory discussed (e.g. Clarke, 2004). A study on polistine wasps demonstrated the problematic nature of this relationship. *P. biglumis*, a paper wasp inhabiting mountainous regions with cool climate, exhibited a considerably lower SMR than *P. dominula* from the warmer lowlands in Austria (Kovac et al., 2020). Kovac et al. (2020) presumed that the colder climate in the mountainous habitat forced these wasps to an energy-saving lifestyle, as the foraging time for resources is restricted due to the harsher climate conditions.

An increased metabolism due to an adaptation to cold climates, as stated by the metabolic cold adaption hypothesis, may be beneficial to insects by enabling them to complete growth, development and reproduction at relatively low temperatures during the breeding season (Chown & Gaston, 1999; Terblanche et al., 2009). However, what does this
mean for insects during overwintering, when they are in a state of dormancy in which metabolic rate is suppressed but still temperature-dependent? Temperature is directly determining the rates of metabolic processes, and higher temperature increases metabolism, and thus consumption of stored energy resources. In the wasps' habitat, the mean annual climatological normal temperature (1971–2000) from Florence in Italy, the region of the Mediterranean wasps, was significantly higher than in the Austrian wasps’ habitat (Kovac et al., 2017). With our representative measurements of the temperatures in the winter hibernacula (Fig. 3) we could demonstrate that the winter temperatures in the Mediterranean micro-habitat were also higher. Although the SMR of the Mediterranean wasps was lower, we calculated a 1.6-fold higher mean mass specific energy turnover for *P. dominula* AT and a 1.7-fold higher for *P. gallicus* IT in comparison to *P. dominula* AT. The higher energy demand is a result of the warmer environment. This means that the Italian species have to be more economical with their resources for a secure and successful overwintering, if they have the same amount of energy stores than the Austrian species. A lower SMR (as we measured in the Italian wasps) reduces the energy demand in any case. This variation of the SMR in closely related species is an example of the consequences of differing environmental conditions on physiological traits. We are aware of the problem of generalization from a single observation, as we measured just one hibernaculum in one season in each species. However, the hibernacula we had chosen for measurements served as appropriate shelter for several consecutive years (personal observation), and the mean annual climatological data confirm our result.

Metabolic measurements are an appropriate tool to assess a species’ vulnerability for climate warming. Our investigation on the metabolism of overwintering gynes showed that metabolic rates are affected not only by mass and reproductive state but also by ecology and lifestyle. This is in good accordance with the ‘Adaptable Informed Resource Use (AIRU) model’ suggested by Glazier (2015b). He calls for a more complex, expanded view, involving multi-directional interactions between the metabolic rate and body size, temperature, biological processes and various ecological factors. He argues that metabolism facilitates many biological processes by providing fuel according to changing need (a demand-driven process), rather than simply controlling rate processes by how much energy and materials are provided (a supply-driven process). In the context of increasing temperatures due to climate change, this expanded view gains special importance because literature reports as well as our own findings show that results may appear controversial because of differing strategies of overwintering. Klockmann & Fischer (2019) found that warmer and

### Table 1.

| Temperature (°C) | SMR mean | SD | N | AMR mean | SD | N | Suppression of MR (W/G) |
|------------------|----------|----|---|----------|----|---|-------------------------|
| **P. dominula AT** |          |    |   |          |    |   |                         |
| 15.0             | 2389.47  | 1486.50 | 196 | 4233.96  | 3867.66 | 54 | 0.56                    |
| 20.0             | 4103.58  | 1249.51 | 69  | 8042.29  | 3734.42 | 79 | 0.51                    |
| **P. gallicus IT** |          |    |   |          |    |   |                         |
| 15.0             | 3246.86  | 2100.18 | 85  | 29593.20 | 20718.23 | 12 | 0.21                    |
| 20.0             | 6101.43  | 2128.69 | 75  | —        | —    | — | —                      |

**Suppression of MR (W/G)**

— Measurements were conducted with the same method and equipment in the same observation period (2017–2019). The suppression of the gynes' metabolic rate (MR) in relation to the workers' MR (worker/gynae) is calculated.

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moister winter conditions substantially decreased survival rates and subsequent development in a temperate butterfly (Lycaena tityrus). Williams et al. (2012b) showed in a study on three Lepidopteran species that they differ in their susceptibility to winter warming. Warmer winters increased energy reserve depletion in Erynnis propertius, but had no effect on the energy reserves of either Papilio glaucus and P. trolus. Higher temperatures during overwintering result in a higher energy demand, and species with relatively high metabolic rates should be more threatened by a premature energy depletion. Therefore, from our study we should expect the northern wasp populations (from Austria) to face a relatively greater risk due to their higher metabolism. An increased metabolic rate due to winter warming decreases the fitness of dormant ecototherms by increasing consumption of stored energy reserves (Williams et al., 2012a). However, from our calculation of the higher mean energy turnover during overwintering of the Italian species we should predict at least a similar risk for the Mediterranean species at increased winter warming. Further studies including more comprehensive microclimate measurements, and comparisons of the energy and water reserves of the wasps at the beginning and at the end of overwintering, are necessary to clarify this question.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Means ± SD of CO2 production rate (mass-specific VCO2 in nL g−1 min−1 and individual VCO2 in nL min−1) in dependence on ambient temperature (T) of resting (SMR) and active (AMR) paper wasp gynes, Polistes dominula AT from Austria and Polistes dominula IT and Polistes gallicus IT from Italy.

Table S2. Statistical details and the fit parameters of fit functions (VCO2 = y0 + A*exp.(R0*T)) of the mass specific standard (SMR) and active (AMR) metabolic rate (VCO2 in nL g−1 min−1) of paper wasp gynes from Austria (P. dominula AT) and Italy (P. dominula IT, P. gallicus IT).

Table S3. Statistical details and the fit parameters of fit functions (VCO2 = y0 + A*exp.(R0*T)) of the individual standard (SMR) and active (AMR) metabolic rate (VCO2 in nL min−1) of paper wasp gynes from Austria (P. dominula AT) and Italy (P. dominula IT, P. gallicus IT).

Table S4. Summary statistics for air temperatures recorded in a hibernaculum of Polistes dominula in Gschwendt (Austria) and a mixed hibernaculum of P. dominula and P. gallicus in Trespiano (Italy) from November 2018 to April 2019.

Table S5. Test statistics of an ANCOVA comparing standard (SMR, Log10 VCO2 in nL min−1) and active metabolic rate (AMR, Log10 VCO2 in nL min−1) of paper wasp gynes. (A) Single values of each individual and (B) average values of individuals.

Table S6. Pairwise comparison with ANOVA of standard metabolic rate (SMR, Log10 VCO2 in nL g−1 min−1) of paper wasp gynes. (A) single values of each individual and (B) average values of individuals.

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