Dung beetle resistance to desiccation varies within and among populations

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Abstract. The study of desiccation resistance and its underlying traits is key to understanding species responses to changes in water availability, especially in the context of predicted increases in the frequency and severity of droughts due to climate change. We performed laboratory experiments using dung beetles, important ecosystem service providers, to investigate variations in physiological traits within species, both at population and individual levels. Desiccation resistance, water loss tolerance and water content were measured in lowland and mountain populations to investigate whether physiological traits vary (i) according to elevation across four species, and (ii) according to sex or male morphology (*minor* and *major* morphs) in two species, and if these responses were consistent across species. Our results showed that desiccation resistance of dung beetles varies both at individual and population levels. We found that desiccation resistance varied between lowland and mountain populations, but no differences were found for other traits such as water loss tolerance. Moreover, differences in individual physiological responses between females, *major* and *minor* males suggest that females were more resistant to desiccation than *minors* and *majors*, but these responses were species-dependent. Our analysis at two hierarchical levels, individual and population, emphasizes the importance of considering within-species variability in predictions of how species may respond to future climatic conditions. Predictions of the responses of species to environmental change may produce different conclusions if they rely on observations from single populations or take into account only a limited range of phenotypes per population.

Key words. Alps, climate change, intraspecific variability, physiological responses, predictions, tunnelers.

Introduction

Insects are threatened by changes in precipitation patterns under climate change, because of their high surface area-to-volume ratio, proportionately low fat storage and relatively high metabolic rate (Gibbs *et al.*, 2003; Harrison *et al.*, 2012; Cardoso *et al.*, 2020). Desiccation resistance has been shown to be a useful trait whose study can lead to more general inferences about the response of species to changes in water availability (Dias *et al.*, 2013), especially in the light of predicted increases in the frequency and severity of droughts (IPCC, 2014). Desiccation

resistance in insects is mainly explained by decreasing rates of water loss (i.e. water lost per hour) which in turn are determined by body size (e.g. Tsai *et al.*, 1998; Harrison *et al.*, 2012; Dias *et al.*, 2013). Other ways that influence desiccation resistance include storing more water in the body, and in tolerating more extreme levels of dehydration (i.e. water loss tolerance) (Gibbs & Matzkin, 2001). Investigating water loss in insects in a context of environmental change represents a big challenge in physiological ecology, climate change and functional biology, as shown by the increase in relevant studies over the years (e.g. Addo-Bediako *et al.*, 2001; Chown *et al.*, 2011; Tejeda *et al.*, 2014; Bujan *et al.*, 2016; Krupp *et al.*, 2020).

Dung beetles (Coleoptera:Scarabaeoidea) are important providers of ecosystem services such as dung removal, nutrient cycling, plant growth enhancement and reductions

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in greenhouse gas emissions (Beynon *et al.*, 2012; Braga *et al.*, 2013; Nervo *et al.*, 2014; Braga *et al.*, 2017; Nervo *et al.*, 2017; Piccini *et al.*, 2017), and represent useful model organisms to understand the relationship between ecological and physiological variation at intraspecific, interspecific and assemblage levels (Chown *et al.*, 1995; Davis *et al.*, 2008; Gaston, 2009; Chown & Klok, 2011). Desiccation resistance has the potential to shape dung beetle assemblages and affect the distribution of species (Chown *et al.*, 1995; Le Lagadec *et al.*, 1998; Roslin *et al.*, 2009; Giménez Gómez *et al.*, 2018). Moreover, the interplay between desiccation resistance and traits such as body mass can influence body size distribution across species (Chown *et al.*, 1995; Le Lagadec *et al.*, 1998; Chown & Klok, 2003). Generally, large body sizes have higher desiccation resistance due to reductions in water loss rate and fatal water loss as a consequence of reduced surface-to-volume ratio and larger water reserves (Chown *et al.*, 1998; Gibbs *et al.*, 2003; Harrison *et al.*, 2012; Dias *et al.*, 2013).

Evidence suggests that the level of genetic variation for desiccation resistance can vary greatly between species which could have drastic effects on a species' ability to survive climatic change (Kellermann *et al.*, 2006). Variations in physiological variables have been mainly investigated at an interspecific level in insects, while less work has been done at an intraspecific level (Gaston & Blackburn, 2000; Albert *et al.*, 2010; Hulshof & Swenson, 2010; Stillwell *et al.*, 2010). Within-species variations in physiological responses of organisms may be determined by differences in body mass, for example between males and females (Renault & Coray, 2004; Kwan *et al.*, 2008), but also by other traits such as cuticular lipid composition (Gibbs, 2002a) or metabolic rates (Hoffmann & Parsons, 1993). Furthermore, physiological responses may vary significantly within horned dung beetle species (e.g. Lailvaux *et al.*, 2005) where males are dimorphic with respect to horn expression, with large males expressing disproportionately long horns (*major* morphs), whereas smaller males remain hornless or express greatly reduced horns (*minor* morphs) (Moczek & Emlen, 2000; Macagno *et al.*, 2009). However, little is known about the links between intraspecific variation in male armaments and physiological variables such as metabolic rates (Lailvaux *et al.*, 2005).

Desiccation resistance and underlying traits such as water loss rate, and water loss tolerance have also been shown to be influenced by habitat type (Chown & Klok, 2011). In general, numerous studies have shown or argued that metabolic rates in species or populations from dry environments are lower than those in species or populations from more mesic environments, thus reducing water loss under xeric conditions (Juliano, 1986; Hoffmann & Parsons, 1989; Gaston & Chown, 1999; Davis *et al.*, 2000; Gibbs, 2002b; Gibbs *et al.*, 2003). While many arguments have been raised regarding the effective contribution of respiratory transpiration to total water loss, large-scale comparative studies (Zachariassen, 1996; Zachariassen *et al.*, 1987; Addo-Bediako *et al.*, 2001; Gibbs *et al.*, 2003) strongly suggest that respiratory transpiration must account for a significant proportion of water lost by arid-environment insects during dehydration.

Water availability represents a key factor influencing species distributions (McCain & Colwell, 2011), and it is implicated, across taxa, in generating stronger selection gradients than temperature (Siepielski *et al.*, 2017). Studies on environmental gradients facilitate the understanding of biotic and abiotic factors that influence species distributions. Some of the steepest environmental transitions in nature are found between different elevations in mountains, where abiotic factors such as temperature, water availability, humidity, solar radiation and partial oxygen pressure change rapidly across the gradient (Slatyer & Schoville, 2016). Exploring how populations respond to macroclimatic variations along elevation gradients provides a test of consistency in response across species, and thus the extent to which generalizations about current and future impacts of climate change in mountain areas can be made (Menéndez *et al.*, 2014). According to the 'climatic variability' hypothesis, to survive at higher elevations, individual organisms need to be able to withstand a broader range of local climatic conditions than at lower elevations. As a consequence, the species more able to withstand wide climatic extremes may be more likely to be adapted to climate change (e.g. Allee *et al.* 1949; Dobzhansky 1950; Janzen 1967; Addo-Bediako *et al.*, 2000).

In this study, we performed laboratory experiments by using dung beetles belonging to the tunneler functional group, that are important ecosystem service providers, to investigate variations in physiological traits within species at two levels, the population and the individual. We posed the following questions: (i) does the response to desiccation resistance vary according to elevation (population level), and is this response consistent across species; (ii) does the response to desiccation resistance vary according to sex or male morphology within species (individual level)?

Material and methods

Species collection and trait measurements

Individuals of four dung beetle species were collected in September 2019 in three different areas in Piedmont, north-west Italy (Fig. 1). All four species were collected from the pastures of the Istituto per le Piante da Legno e l'Ambiente (IPLA) in Torino (45°05′18.5″N, 7°44′28.5″E, 300 m a.s.l.). Two of the four species (*Geotrupes stercorarius* and *Onthophagus fracticornis*) were also collected in the pastures around the sanctuary of S. Anna di Vinadio (44°13′55.2″N, 7°06′18″E, Stura Valley, 2035 m a.s.l.). The other two species (*Onthophagus taurus* and *Euoniticellus fulvus*) were collected in the pastures of Demonte (44°18′59″N, 7°17′59″E, Stura Valley, 780 m a.s.l.). Sampling areas were from two different climatic regions: the Po Plain (Torino) which has a continental climate characterized by warm summers (temperatures often exceeding 30°) and cold winters, and the Alpine region (Demonte, S. Anna di Vinadio) which has a cold temperate climate with relatively high precipitation (Supporting information, Table S1) (Fratianni & Acquaotta, 2017). Species, collected in cattle and sheep dung, belonged to one functional group (i.e. tunnelers) defined according to reproductive strategy (Table 1).
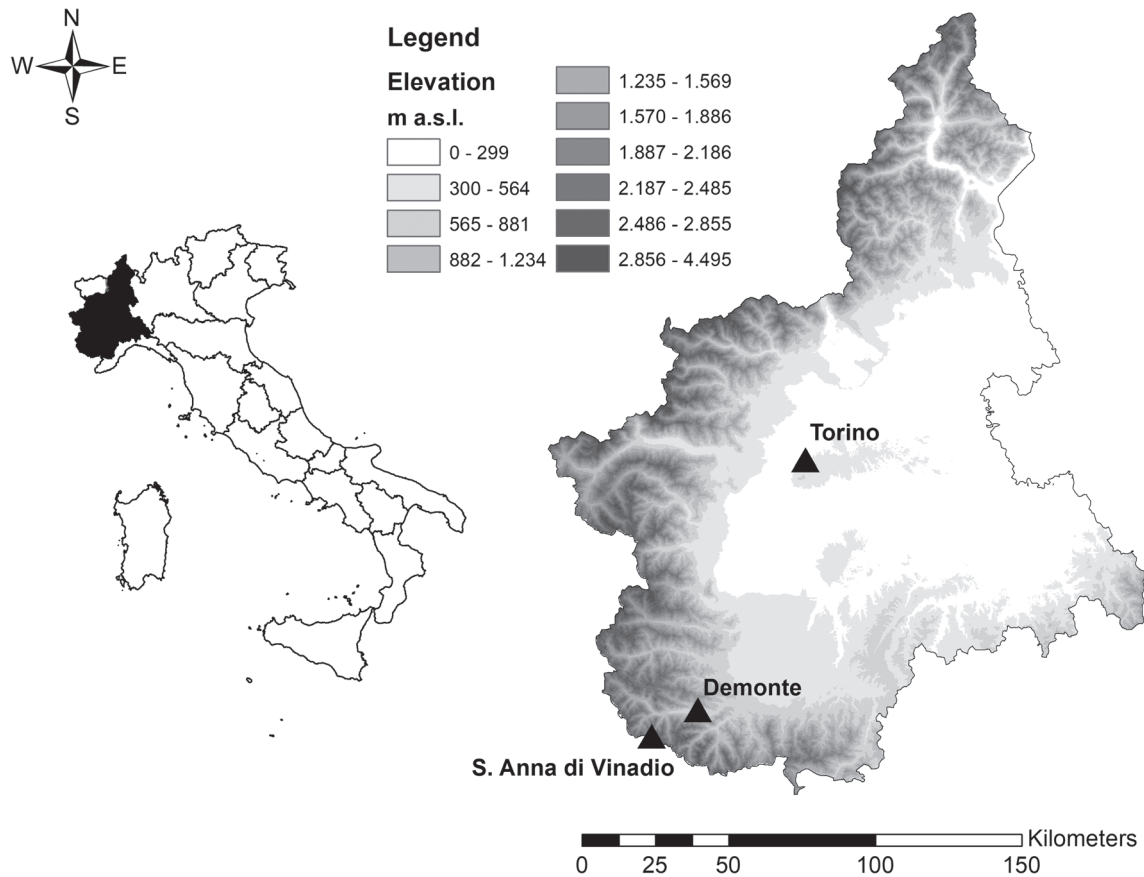
Fig. 1. The three sampling areas where dung beetle species were collected representing low (Torino) and high (Santa Anna di Vinadio, Demonte) elevation populations

Table 1. Species collected in the three sampling areas: Torino, Demonte and Sant'Anna di Vinadio (S. A. V.). All the species have a similar reproductive strategy (tunnelers): they dig tunnels below the dung mass in which they bury brood balls.

		Population level			Individual level		
		Lowland sites	Mountain sites				
Family	Species	Torino	Demonte	S.A.V.	*Major* M	*Minor* M	Females
Geotrupidae	*Geotrupes stercorarius (Linnaeus, 1758)*	8 (4)		30 (27)			
Scarabaeidae	*Onthophagus fracticornis (Preyssler, 1790)*	9 (2)		30 (10)	10	11	18
	Onthophagus taurus (Schreber, 1759)	9 (3)	7 (5)		6	7	3
	Euoniticellus fulvus (Goeze, 1777)	7 (4)	8 (4)				

The number of individuals of each species used in the experiment is specified, as is the number of individuals of *O. fracticornis* and *O. taurus* for each sex and morphology category (*majo*r and *minor* males – M), and females. The numbers in parenthesis indicate the number of dead individuals. The overall sample size was 108 individuals.

We followed the trait-based protocol suggested by Moretti *et al.* (2017) for standardized measurement of traits in terrestrial invertebrates that has the potential to serve as a basis for comparative studies using functional traits (Bertelsmeier, 2017). Before exposing beetles to dry conditions and measuring desiccation resistance, an acclimation procedure to replenish any possible water deficit in the collected dung beetles was carried out. Beetles were kept isolated in small cylinders (diameter 2 cm, height 3 cm) placed in a closed glass box ($40 \times 20 \times 20$ cm³), on top of a 3 cm layer of moist floral foam for 3 days, ensuring

constant conditions of 100% relative humidity (RH). The laboratory was kept at a temperature of 20 °C for the whole acclimation period (average temperature: 20.4 ± 0.3 °C) under an LD 12:12 h. The cylinders were open at both sides which were covered with a nylon mesh cloth (width 0.5 mm) to prevent beetles from escaping, but allowing an adequate airflow through the cylinder. This pre-treatment procedure was done without food to control for water loss from excretion. After this procedure, insects were exposed to realistic dry conditions, approximately 70% RH, to record water loss rate, and percentage of

fatal water loss. This RH value was chosen to represent a moderate stress condition considering that, on average, cattle dung has a relative humidity of 80%, and that 91–93% is the threshold above which arthropods (e.g. terrestrial isopods, beetles) are able to absorb water vapour (Wright & Machin, 1990; Hansen *et al.*, 2006). The humidity level of 70% RH was reached using a glycerol–water solution in volume concentration of 48% (sensu Dias *et al.*, 2013), and measured with humidity data loggers (Plug & Track Plug & Track™). Plastic glasses (200 mL) were filled with 80 mL of glycerol solution. In each glass, a platform made of iron wire was placed about 1 cm above the solution's surface; the cylinder containing the animal was then placed on this platform and the glass closed with a plastic cover. The cylinders were open at both sides and covered with a nylon mesh cloth to prevent beetles from escaping, but allowing an adequate airflow between the solution and the cylinder inside the glass. The temperature range for the experimental period was 21–22 °C, under an LD 12:12 h.

We measured 7–30 individuals per species group depending on the abundance of the animals collected in the field. Individuals of *O. fracticornis* and *O. taurus* were measured to investigate intraspecific variations in physiological responses based on sex and male dimorphism (e.g. 3 sex/morphs categories: *minor* males, *major* males and females) (Table 1). Males of *O. fracticornis* and *O. taurus* were classified as *major* or *minor* morphs based on horn presence and head shape (Macagno *et al.*, 2009).

Before exposing the beetles to dry conditions, individuals in each cylinder were weighed to record the initial fresh mass using an analytical balance (Precisa 125 A, 0.1 mg). We recorded changes in individual body mass (mg) every day at four time points (i.e. 9.00, 12.00, 15.00, 18.00 h). Before weighing the dung beetles, we checked if they were alive by disturbing them gently with a soft brush or by flipping them with tweezers. By weighing the cylinders containing the beetles at regular times, we were able to register weight changes, and, at the same time, minimize disturbance. The test was conducted for 96 h, after which we ended the experiment even if some individuals were still alive, to avoid other potential stress factors, such as starvation, influencing the measurements (Moretti *et al.*, 2017). Once the experiment was over, we measured the dry body mass. To measure the dry body mass (hereafter body mass), the insects were dried and weighed until the weight was constant between subsequent measurements.

We measured three different physiological response traits: desiccation resistance, water content and fatal water loss. *Desiccation resistance* was estimated for all the individuals as the inverse of water loss rate (Harrison *et al.*, 2012). Water loss rate describes the rate of water loss from an individual over a given period of time (proportion of initial water content lost per hour, i.e. mg mg^{-1} hr^{-1}). Water content, which is the maximum water content that an organism is able to store, was expressed as *fractional water content* (initial water content/dry body mass). Initial water content was calculated as the initial wet body mass – dry body mass. The *percentage of fatal water loss* (hereafter *water loss tolerance*) was calculated only for dead individuals (Table 1) and was expressed as the proportion of the initial water content that was lost at the time of death i.e. [(initial wet body mass – final wet body mass)/initial water content] × 100.

If the individual died overnight, we used the average value of body mass of the last measurement in the afternoon and the first in the morning.

Statistical analyses

The goal of the analysis was to determine the extent to which elevation (population level) and sex/male dimorphism (individual level) influence physiological response variables, and whether these responses are consistent across species.

The effect of elevation on physiological traits (population level). The sampling sites were divided into two elevation categories with different climatic conditions (Supporting information, Table S1): lowland sites (Torino) and mountain sites (Demonte and Sant'Anna di Vinadio). The interaction effect between elevation category and species (i.e. *E. fulvus*, *G. stercorarius*, *O. fracticornis*, *O. taurus*) on desiccation resistance, water loss tolerance and water content was tested by using Generalized Linear Models (GLM) with Gamma (for desiccation resistance and water content), and Binomial (for water loss tolerance) distributions. Visual inspection of frequency distributions and Shapiro–Wilk tests confirmed the non-normality of errors. We checked for interactions and non-linear terms between the covariates. If the interaction effect was not significant, the term was added to the model as an additive factor. We removed non-significant terms from the model.

The effect of sex and male dimorphism on physiological traits (individual level). The effect of sex and male dimorphism (i.e. 3 sex/morphs categories: *minor* males, *major* males and females) on physiological responses was tested for *O. fracticornis* and *O. taurus* with separate models for each species. Individual variation was tested within species, since the two *Onthophagus* species showed species-specific responses in the population level models (see Section 3). We ran GLM models with a two-way interaction between sex/morphs and elevation. As mentioned earlier, we used GLMs with a Gamma distribution (desiccation resistance and water content). Here, water loss tolerance was not included in the analysis because not enough individuals in each sex/morph category died. In addition, a GLM with a Gaussian distribution was used to test the differences in dry body mass (log-transformed) among the three sex/morphs to check if differences in physiological responses could be explained by differences in body mass.

The statistical power of our analysis was calculated using the program G*POWER 3.1 (Faul *et al.*, 2009) to ensure that there were not any false-positive results (Forstmeier *et al.*, 2017). We calculated the statistical power (Post hoc analysis – see Cohen, 1988) as a function of significance level ($\alpha = 0.05$), sample size and effect size. G*POWER 3.1 provided power analysis procedures for fixed-predictor models based on GLM; we therefore used the 'Linear multiple regression: Fixed model, R^2 deviation from zero' procedure for *F* tests. Cohen's f^2, the ratio of explained variance and error variance, served as the

effect size measure (Cohen, 1988), and was calculated directly from the squared multiple correlation. The convention to use $\alpha = 0.05$ is quite strong, and Cohen (1988, 1992) suggests power = 0.80 as a sensible goal for research.

Results

Elevation

Desiccation resistance significantly differed between species and elevation (Fig. 2a), while water loss tolerance only differed between species (Fig. 2b, Table 2). Desiccation resistance and water loss tolerance were significantly greater in *O. fracticornis* compared to the other three species (with the exception of water loss tolerance for *E. fulvus*). *O. taurus* was more resistant to desiccation than *E. fulvus*, but this latter species showed greater tolerance to water loss. *G. stercorarius* showed limited water loss tolerance compared to the other three species. Mountain populations of the four species were generally less resistant to desiccation than lowland populations. There was no significant difference in water content between species, but the mountain population of *O. fracticornis* seemed to have greater water content than the lowland population (Fig. 2c). The statistical power of the three models (i.e. desiccation resistance, water loss tolerance and water content) was greater than the threshold of 0.8, suggesting a low probability of a false-positive result. Values of statistical power and effect size are indicated in Table 2. Average trait values and standard deviations for each species and elevation can be found in Supporting information, Table S2.

Sex and male dimorphism

Desiccation resistance was significantly different between sex/morph categories in *O. fracticornis*, but it was not modulated by elevation, as shown by the non-significant interaction (Table 3). Females were more resistant than *major* and *minor* males (Fig. 3a). We found a significant effect of elevation on water content in *O. fracticornis*: *minor* males belonging to the mountain population were found to have higher water content than the lowland population (Fig. 3b). The statistical power of these models was greater than the threshold of 0.8. Values of statistical power and effect size are indicated in Table 3. Regarding *O. taurus*, desiccation resistance was modulated by sex/morph categories and elevation, with *minor* males from mountain populations less resistant compared to the lowland population. We found no significant differences in water content between sex/morph categories and elevation (Table 3). However, it is worth noting that the statistical power of this model was low (0.54) because of the small sample size. These results need therefore confirmation in future studies by using larger sample size. In both species, *major* males and females showed similar body mass, while minor males were significantly smaller (Supporting information, Table S3, Supporting information, Figure S1).

Discussion

Our results showed that desiccation resistance of dung beetles varies both at individual and population levels. Intraspecific variations among populations collected in sites at different elevations, and among individuals with different sexes, seemed to significantly affect the ability of organisms to resist reductions in water availability. We found that desiccation resistance varied between lowland and mountain populations, but no differences were found for other traits such as water loss tolerance. Moreover, differences in individual physiological responses between females, *major* and *minor* males suggest that females were more resistant than *minors* and *majors*, but these responses were species-dependent.

Intraspecific variations of traits among populations collected at different elevations

A moderate amount of variation in desiccation resistance was observed within and between the four species examined. Our results highlighted the importance of elevation in influencing the capacity of a species to buffer environmental change. Indeed, due to within-species variations in desiccation resistance along the elevation gradients, the species as a whole is more likely to have the capacity to adapt to changing conditions. Even though differences in desiccation resistance between lowland and mountain populations to changes in moisture availability included all the four species, they seemed more evident for *G. stercorarius*. The mountain populations seemed to be more sensitive than the lowland ones. On the contrary, results for water loss tolerance highlighted significant differences among species, but no differences between mountain and lowland populations. The higher resistance in the mountain populations of the four species was combined with higher reserves of body water content only for *O. fracticornis*. Populations of the other three species were characterized by similar reserves of body water. These results seem to suggest that differences in desiccation resistance are not only caused by difference in initial water content (as also shown in Nghiem *et al.*, 2004); there were other factors that influenced desiccation resistance and water loss between lowland and mountain populations, for example differences in metabolic rates (Addo-Bediako *et al.*, 2001), or in morphological and anatomical parts that may represent potential weak points for water loss (e.g. spiracle and wing size) (Arcaz *et al.*, 2016; Pass, 2018).

We found that related species such as *O. fracticornis*, *O. taurus* and *E. fulvus* belonging to the Scarabaeidae family were characterized by high levels of resistance (i.e. *O. fracticornis*, *O. taurus*) or tolerance to water loss (i.e. *O. fracticornis*, *E. fulvus*). The Scarabaeidae family constitutes the main thermophilous group of dung beetles (Lumaret & Kirk, 1991; Lobo & Martín-Piera, 2002; Lobo *et al.*, 2002); its ability to resist and tolerate arid conditions may be linked to anatomical and morphological characteristics that require further investigations. As a caveat, we should highlight that starvation in the pre-treatment procedure may decrease metabolic rates and activities of insects, resulting in changes to cuticle and respiratory
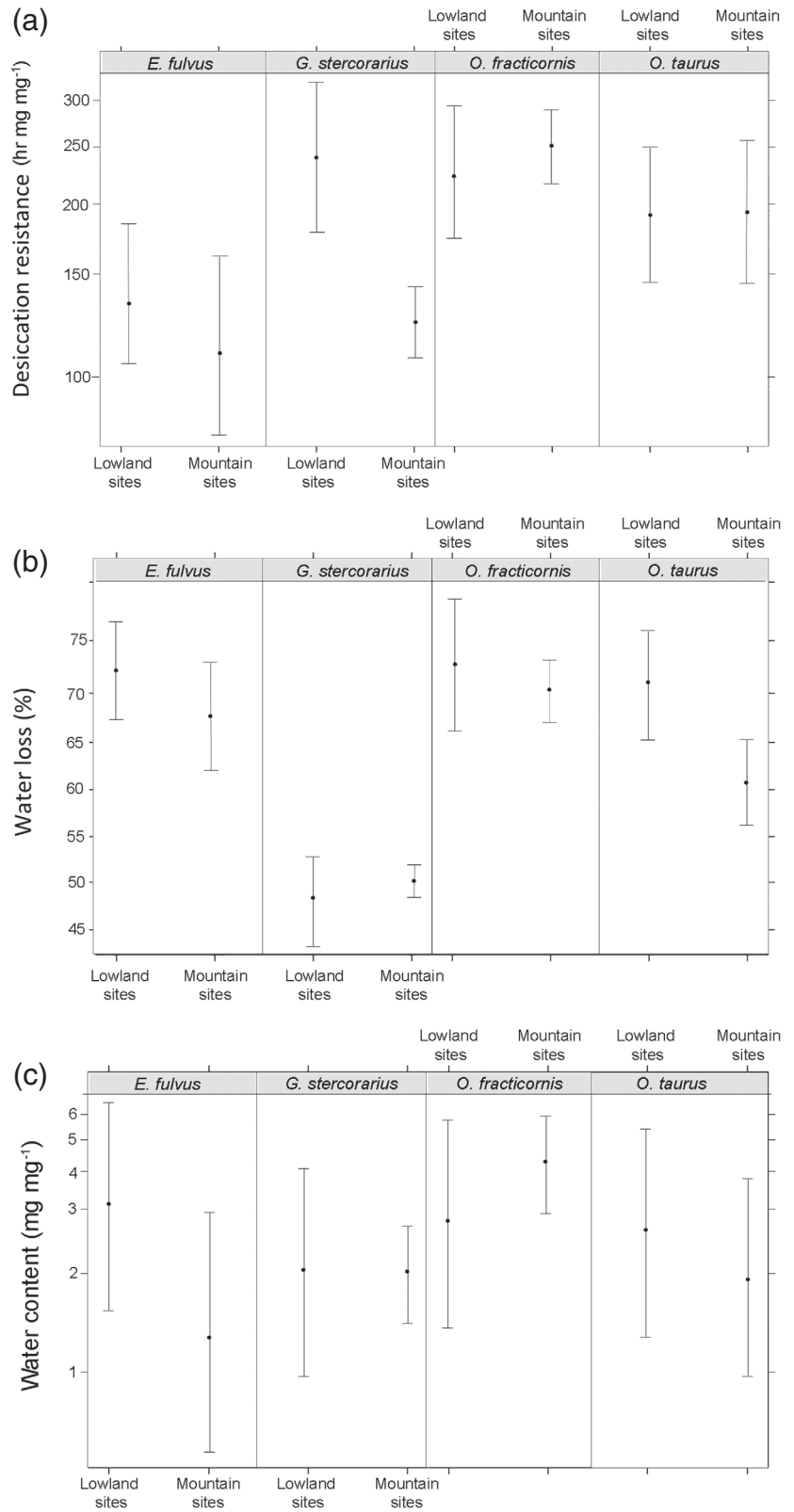
Fig. 2. Differences between species and elevation in terms of desiccation resistance, water loss tolerance and water content are represented, respectively, in (a), (b) and (c). 95% confidence intervals are displayed around each fitted line

Table 2. Full models regarding the effect of elevation on physiological response variables such as desiccation resistance (DR), water loss tolerance (WLT) and water content (WC).

Generalized linear model (GLM) (sample size = 104, effect size = 0.32, statistical power = 0.97)

Reference level: *Euoniticellus fulvus*

DR ~ Elevation + Species

	Estimate ± Standard error	t value	P
Intercept	4.923 ± 0.127	38.856	0.000
Geotrupes stercorarius	0.203 ± 0.143	1.414	0.160
Onthophagus fracticornis	0.759 ± 0.142	5.358	0.000
Onthophagus taurus	0.426 ± 0.157	2.702	0.008
Mountain sites	−0.204 ± 0.094	−2.171	0.032

GLM (sample size = 56, effect size = 0.36, statistical power = 0.99)

WLT ~ Species

	Estimate ± Standard error	z value	P
Intercept	0.827 ± 0.082	10.067	0.000
G. stercorarius	−0.789 ± 0.089	−8.806	0.000
O. fracticornis	0.055 ± 0.105	0.523	0.601
O. taurus	−0.235 ± 0.110	−2.130	0.033

GLM (sample size = 104, effect size = 0.30, statistical power = 0.99)

WC ~ Elevation * Species

	Estimate ± Standard error	t value	P
Intercept	1.137 ± 0.359	3.166	0.002
G. stercorarius	−0.445 ± 0.508	−0.876	0.383
O. fracticornis	−0.106 ± 0.479	−0.222	0.824
O. taurus	−0.156 ± 0.479	−0.327	0.744
Mountain sites	−0.894 ± 0.556	−1.607	0.111
G. stercorarius* Mountain sites	0.915 ± 0.684	1.337	0.184
O. fracticornis* Mountain sites	1.310 ± 0.663	1.975	0.050
O. taurus* Mountain sites	0.583 ± 0.723	0.806	0.422

water loss (Rolandi *et al.*, 2014). Difference in metabolic rates at the species level is therefore an extra-factor that may cause interspecific differences in water loss.

Making predictions about resistance to climate change across and within species is complicated by the fact that different species have different geographical distributions. For example, distributions of *O. fracticornis* and *G. stercorarius* reach higher elevations than *O. taurus* and *E. fulvus* (Negro *et al.*, 2011, 2011b; Tocco *et al.*, 2013, 2013b). Moreover, the relationship between elevation and desiccation resistance is complex, especially because desiccation rate can be strongly influenced by temperature (Addo-Bediako *et al.*, 2001). For example, some species show temperature-dependent survival after desiccation stress (Worland & Block, 1986) and temperature-dependent humidity preferences (Hayward *et al.*, 2001). In this regard, the desiccation resistance–elevation relationship is not uniform among studies: some studies show that desiccation resistance decreases with elevation (Addo-Bediako *et al.*, 2001; Chown *et al.*, 2011), while others on *Drosophila* sp. have found an increasing desiccation resistance with elevation (e.g. Parkash *et al.*, 2008). Organisms at higher elevations (or latitudes) are expected to be able to withstand more variable and extreme

conditions, thus having a wider range of physiological tolerance and plasticity (Janzen, 1967; Seebacher *et al.*, 2015), in accord with the 'climatic variability' hypothesis. This hypothesis was originally developed to explain biodiversity patterns in tropical mountains, but was then generalized to different ecosystems and taxa (e.g. Gaston & Chown, 1999; Calosi *et al.*, 2010; Pintor *et al.*, 2015; Sheldon *et al.*, 2015; Beck *et al.*, 2016; Gutiérrez-Pesquera *et al.*, 2016; Mumladze *et al.*, 2017). The strongest selection for broader climatic tolerances is supposed to be in the areas with the greatest annual range of climatic conditions, such as mountain areas that are characterized by higher variability in climatic conditions than lowland areas (Rasmann *et al.*, 2018). Contrary to expectations, mountain areas in our study showed lower variability in climatic conditions than lowland areas, especially in terms of difference between minimum and maximum precipitation rates (Supporting information, Table S1). In this regard, we found that populations from mountain areas were more sensitive to desiccation than from lowland areas. Our findings therefore support the hypothesis that species exposed to greater climatic variability evolve broader physiological tolerance (Seebacher *et al.*, 2015), but the tolerance did not necessarily increase with elevation. This may have different

Table 3. Full models regarding the effect of sex and male dimorphism on the physiological response variables desiccation resistance (DR), water loss tolerance (WLT) and water content (WC).

Onthophagus fracticornis				Onthophagus taurus			
GLM (SS = 37, ES = 0.23, SP = 0.81)				GLM (SS = 15, ES = 0.49, SP = 0.54).			
Reference level: minor males				Reference level: minor males			
DR ~ Sex/morphs				DR ~ Sex/morphs * Elevation			
	Estimate ± SE	*t* value	P		Estimate ± SE	*t* value	P
Intercept	5.370 ± 0.129	41.460	0.000	Intercept	5.331 ± 0.154	34.667	0.000
Females	0.340 ± 0.160	2.126	0.041	Females	−0.064 ± 0.377	−0.170	0.869
Major M	−0.163 ± 0.188	−0.868	0.391	*Major* M	−0.237 ± 0.288	0.826	0.428
				Lowland sites	−0.881 ± 0.288	−3.063	0.012
				Females * Lowland sites	0.817 ± 0.510	1.603	0.140
				Major M * Lowland sites	1.254 ± 0.414	3.029	0.013
GLM (SS = 37, ES = 0.40, SP = 0.91)				GLM (SS = 15)			
WC ~ Sex/morphs * Elevation				WC ~ Sex/morphs			
	Estimate ± SE	*t* value	P		Estimate ± SE	*t* value	P
Intercept	1.895 ± 0.160	11.806	0.000	Intercept	0.830 ± 0.160	5.178	0.000
Females	−0.569 ± 0.188	−3.028	0.005	Females	0.259 ± 0.292	0.884	0.323
Major M	−0.748 ± 0.218	−3.418	0.002	*Major* M	−0.098 ± 0.236	−0.417	0.683
Lowland sites	−0.821 ± 0.254	−3.235	0.003				
Females * Lowland sites	0.332 ± 0.354	0.936	0.356				
Major M * Lowland sites	0.868 ± 0.405	2.145	0.040				

SS = sample size, ES = effect size, SP = statistical power.

causes, for example, rain and clouds at high elevation occur all year round, therefore the generally higher amount of rainfall in Alpine areas may have selected for individuals with a narrow tolerance to desiccation (Addo-Bediako *et al.*, 2000), while the accentuated seasonal variations in temperature and precipitation in lowland areas may have selected for organisms more prone to survive in conditions of unfavourable humidity. Our study suggests that the physiological responses of a species to changes in climatic conditions may vary if we consider different populations of the same species. Generalizations about current and future impacts of climate change for a given species may be inaccurate or limited if observations are restricted to a single population (Matzkin *et al.*, 2007; Chen *et al.*, 2011). For example, if we tested only the lowland population of *G. stercorarius*, we would have concluded that this species was more resistant than it actually was. However, we must acknowledge some limitations of our experimental analysis at the population level, which were (i) the limited number of species investigated, (ii) the limited number of sampling sites and (iii) the lack of microclimatic measurements in the sampling sites. Future studies should focus on those species whose distribution encompasses large elevation gradients, and should consider more regions thus enabling more robust generalizations.

Despite the limitations of our study, we propose that differences in physiological responses between populations may be linked to specific strategies aimed at reducing water loss that need further investigation. Previous studies on species from mesic and xeric environments have reported that lower rates of water loss in the xeric species are achieved primarily by reduction in respiratory losses associated with a reduction in metabolic rate and activity levels, and improved spiracular control (Duncan & Byrne, 2000; Chown & Davis, 2003). This hypothesis has received considerable experimental support for Coleoptera (Duncan & Byrne, 2000; Chown & Davis, 2003), and Hymenoptera species (Schilman *et al.*, 2005), but it has found less support for other taxa (e.g. Orthoptera: Acrididae – Huang *et al.*, 2015). An important role in regulating respiratory water loss in beetle species from xeric and mesic environments seems to be played by the sub-elytral chamber. In mesic beetle species, it seems that the largest contribution to gas exchange is made by the abdominal spiracles within the sub-elytral chamber, while in xeric species, the contribution made by mesothoracic spiracles increases substantially (Duncan & Byrne, 2005). It appears that gas exchange through mesothoracic spiracles, especially the right one which is equipped with a sieve plate, reduces the cross-sectional area over which exchange takes place, thus lowering water loss (Duncan & Byrne, 2002).

Finally, a higher sensitivity of Alpine dung beetle populations belonging to some tunneler species (i.e. *E. fulvus*, *G. stercorarius*, *O. fracticornis* and *O. taurus*) is especially concerning considering that warming in the Alps is projected to be 1.5 °C in the first half of 21st century, and lower precipitation is expected in the summer (Gobiet *et al.*, 2014). Tunnelers which dig tunnels below the dung mass in which they bury brood balls are considered important ecosystem service providers, especially in mountain pastures (Nervo *et al.*, 2017). Species belonging to this nesting strategy have been shown to have large short-term effects on dung removal rates and other functions such as nutrient
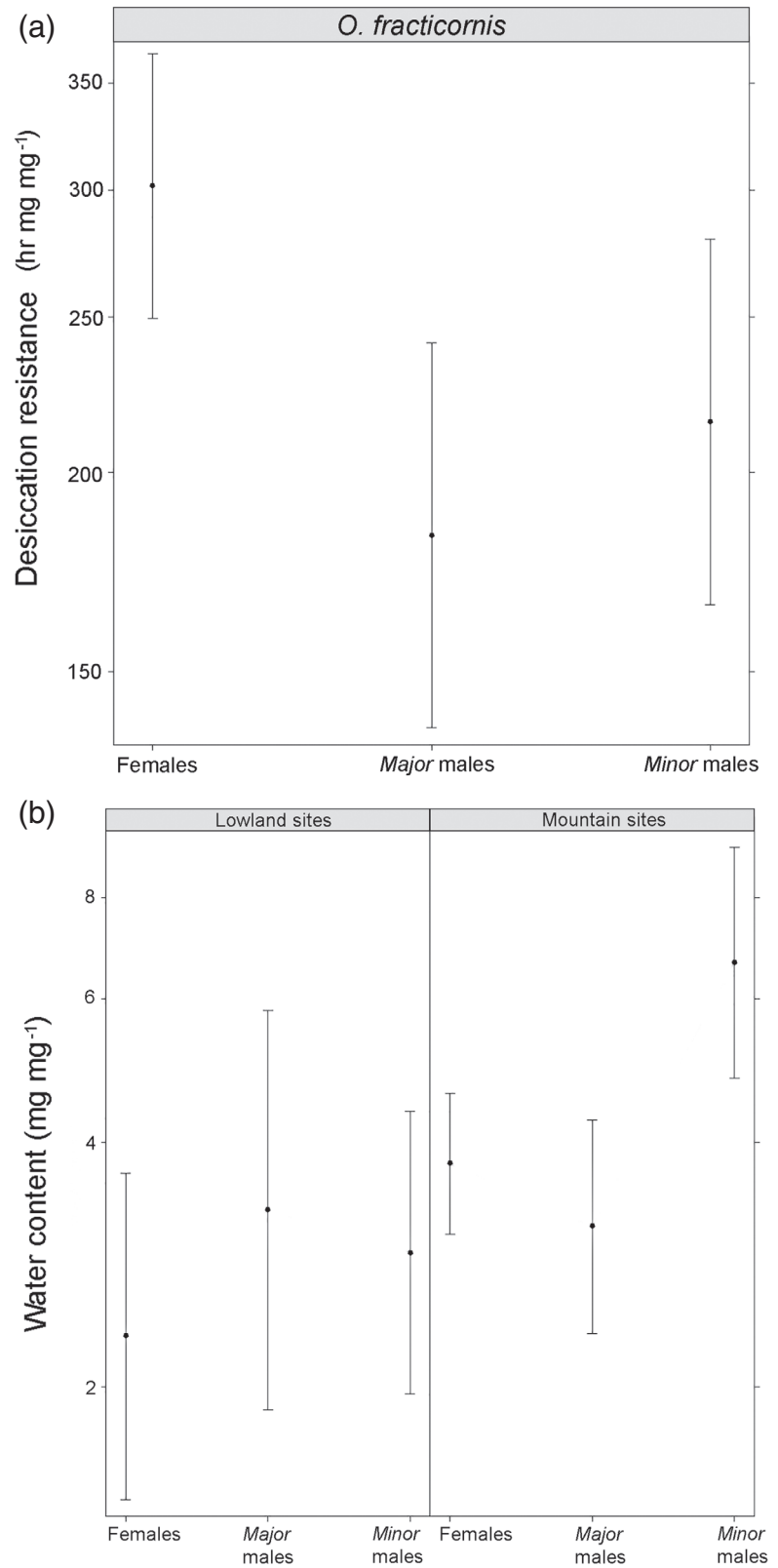
Fig. 3. Differences in desiccation resistance among sexes and male dimorphism in *O. fracticornis* (a), and the interaction effect between sex/male dimorphism and elevation on water content (b). 95% confidence intervals are displayed around each fitted line

cycling, and plant growth enhancement (Nervo *et al.*, 2017), with larger species having a disproportionate effect (Rosenlew & Roslin, 2008; Nervo *et al.*, 2014). The higher sensitivity to dry conditions of the mountain population of large tunnelers such as *G. stercorarius* opens important questions not only regarding the conservation of the species, but also about the possible functional consequences of changes in dung beetle communities for ecosystems.

Intraspecific variations of traits among dimorphic males and females

Previous ecological studies have shown the importance of considering not only functional diversity at a species level, but also at an individual level (Pruitt & Ferrari 2011; Palacio *et al.*, 2019), given that intraspecific variability between individuals is at the base of the adaptation of organisms to environmental changes (Hallgrímsson & Hall, 2005; Dall *et al.*, 2012). For example, males and females of the same species may experience differential or even opposing selection pressures in changing environments (Kwan *et al.*, 2008). If on the one hand the role of intraspecific variations needs more attention in ecological studies (Albert *et al.*, 2012; Violle *et al.*, 2012; Des Roches *et al.*, 2018), on the other, to the best of our knowledge, little has been done on the role of sex and dimorphic males within dung beetle species.

Our results showed that females in *O. fracticornis* were more resistant to desiccation than *major* and *minor* males, and these differences were consistent in lowland and mountain populations. Differences between sexes were not found for *O. taurus*, but this may be probably linked to the small sample size for *O. taurus* populations, as also shown by the low statistical power of the results that therefore will not be discussed in detail here. Results found for *O. fracticornis* are in line with other studies on different taxa (e.g. Tenebrionidae; Drosophilidae – Renault & Coray, 2004; Kwan *et al.*, 2008), that showed higher resistance to desiccation in females. Storage of a large amount of water might be advantageous in extending the duration of survival during dry periods (Harrison *et al.*, 2012), but no significant differences occurred between the water content of males and females. Morphological adaptations, such as increased body size, have been found to significantly reduce water loss (Edney, 1977) due to a proportional decrease in the surface area with increasing size (Hadley, 1994). Body size might explain differences in resistance between females and *minor* males, as females were significantly larger than *minors*. However, body size did not explain differences between females and *majors*, since they showed similar sizes. *Minor* and *major* males showed similar desiccation resistance even though *minors* were significantly smaller than *majors*. These results suggest that body size is not the only factor explaining resistance to desiccation among sexes and morphs. Desiccation resistance is also highly dependent on the ability to tolerate water loss. Previous studies suggested that females were able to tolerate higher loss of body water than males because of larger amounts of lipid reserves (Beenakkers *et al.*, 1985; Pullin, 1987). Unfortunately, in our study, we were not able to evaluate differences in water loss tolerance between sexes

and morphs because not enough individuals belonging to the three sex/morph categories died. In this regard, we suggest for future studies to test different levels of humidity encompassing values from 0 to 70% to accelerate the death of individuals and assess consistency of responses under different experimental conditions. We considered 70% as a relevant desiccation stressor for dung beetles, since 85% humidity has been suggested to be an appropriate RH for insects inhabiting soil (Dias *et al.*, 2013; Shapiro-Ilan *et al.*, 2014; Moretti *et al.*, 2017). However, dung beetles are flying arthropods that for short periods can be exposed to lower humidity.

Our results suggest that individual composition (i.e. sex) within a population may be a relevant factor influencing the capacity of a species to respond to changes in hydric conditions. In this regard, our population-level models can be considered a rough approximation, especially for *Onthophagus* species that have greater morphological diversity (e.g. females, *major* and *minor* males). However, it is worth noting that the proportion between sexes and dimorphic males was nearly balanced in both species. Future changes in moisture conditions in Alpine areas can be especially concerning because they may affect survival of dung beetles by influencing the sex ratio. Such changes can cause a reduction in mate availability with consequent possible changes in the strength of sexual selection (Kvarnemo & Ahnesjo, 1996; González-Tokman *et al.*, 2017; House *et al.*, 2019).

In conclusion, our analyses at two hierarchical levels, individual and population levels, emphasize the importance of considering within-species variability in predictions of how species may change under future climatic conditions. Predictions of the responses of species to environmental change can produce different conclusions if they rely on observations from only single populations or take into account a limited range of phenotypes per population.

Supporting Information

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

Table S1 Temperature and precipitation values of the three sampling areas.

Table S2 Average trait values and standard deviations for each species and elevation.

Table S3 Full models regarding the differences in dry body mass between females, *major* and *minor* males.

Figure S1 Differences in dry body mass between females, *major* and *minor* males in *O. fracticornis* and *O. taurus*.

the morphological measurements of dung beetles. The authors declare no conflicts of interest.

Author contributions

Beatrice Nervo: conceptualization, data curation, formal analysis, investigation, writing – original draft, writing – review & editing. Angela Roggero: conceptualization, investigation. Dan Chamberlain: formal analysis, writing – review & editing. Antonio Rolando: writing – review & editing, supervision. Claudia Palestrini: conceptualization, writing – review & editing, resources, supervision.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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