Cold-adapted species in a warming world — an explorative study on the impact of high winter temperatures on a continental butterfly

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Abstract

Increasing evidence suggests that global warming significantly alters the range and phenology of plants and animals. Whereas thermophilous species usually benefit from rising temperatures, the living conditions of taxa adapted to cooler or continental climates are deteriorating. The woodland ringlet butterfly, *Erebia medusa* Fabricius (Lepidoptera: Nymphalidae) is one of the continental species that are supposed to be adversely affected by climate change, especially by rising winter temperatures. Here, we conduct an explorative study on the effects of low, moderate, and high winter temperatures on the pre-adult and adult stages of *E. medusa* in a laboratory experiment. Compared to the two other temperature regimes, the warm winter treatment led to an earlier termination of diapause and higher larval weights at the end of the winter, but significantly lower survival rates. The after-effects of the warm treatment included lower weight of the pupae and adult females, shorter forewings of adult males, and earlier emergence of both adult males and females. In natural environments, which are characterized by a much greater thermal variability and a much higher frequency of soil freeze-thaw events compared to our experiment, the effects of rising winter temperatures might be stronger than in this study. Thus, we conclude that warmer winters pose a non-negligible long-term threat to *E. medusa*.

Introduction

There is increasing evidence that global warming causes range shifts and phenological changes in many plant and animal taxa (Walther et al., 2002; Parmesan & Yohe, 2003). Groups of organisms with many highly specialized species, e.g., butterflies and dragonflies, exhibit particularly fast and strong responses to climatic changes and therefore are good indicators of global change (Parmesan, 2003; Thomas, 2005). Thermophilous species usually benefit from global warming by advancing and prolonging their flight time, producing more generations per year, and extending their ranges polewards and to higher altitudes (Roy & Sparks, 2000; Konvicka et al., 2003; Chen et al., 2011). Taxa adapted to cooler climates, however, often suffer from deteriorating living conditions at the low-latitudinal and low-elevational boundaries of their ranges (Thomas & Lennon, 1999; Konvicka et al., 2003; Hickling et al., 2006). Consequently, they have to move to higher latitudes or elevations. If such movements are not possible, e.g., due to habitat fragmentation, range retractions are the consequence (Warren et al., 2001; Hill et al., 2002; Ott, 2010).

In recent years, many studies were devoted to describe and quantify patterns of distributional changes, but few aimed at identifying the underlying mechanisms (Helmuth et al., 2005). Most experimental studies in the context of global warming have focused on the effects of

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higher summer temperatures (Bale & Hayward, 2010) or on the beneficial effects of winter warming (Battisti et al., 2005; Crozier, 2003, 2004; e.g., Ungerer et al., 1999). In the context of nature conservation, however, the negative population trends of cold-adapted species are of particular concern (Wilson et al., 2007). In Europe, global warming induces increasingly warmer and wetter weather conditions during the winter months (Raisanen et al., 2004; IPCC, 2007). Such climatic conditions during winter are supposed to have adverse effects on the diapause stages of cool-stenothermic insect species (Fartmann & Hermann, 2006; Hahn & Denlinger, 2007; Huey, 2010). Longer frost-free periods may lead to higher energy losses due to accelerated metabolism, or to increased fungal and parasite infestation (Fartmann & Hermann, 2006). However, little proof for this assumption has been furnished until today. Evidence for the beneficial effects of low temperatures on hibernating insects was found in the gall fly [*Eurosta solidaginis* (Fitch)] and the rose galling wasp [*Diplolepis spinosa* (Ashmead)] (Irwin & Lee, 2000, 2003; Williams et al., 2003). Individuals of these species that had experienced higher temperatures during diapause showed higher metabolic rates and decreased survival and fecundity. Workers of the wood ant (*Formica aquilonia* Yarrow), which experienced constant winter temperatures around 7 °C, consumed a greater portion of their body fat resources and suffered from a higher mortality than individuals that were kept at 1 °C (Sorvari et al., 2011). Experiments with butterflies showed that winter diapausing adults of the small tortoiseshell [*Aglais urticae* (L.)] and the peacock [*Inachis io* (L.)] incur higher mortality when kept at 10 °C compared to 2 °C (Pullin & Bale, 1989). Similarly, higher rearing temperatures caused greater weight loss and higher mortality in overwintering pupae of the Canadian tiger swallowtail (*Papilio canadensis* Rothchild & Jordan) (Mercader & Scriber, 2008).

Although butterflies are one of the best studied invertebrate groups, our knowledge of the mechanisms which determine their climate niches is still incomplete. This is especially true for the possible climatic causes of population declines of species with continental distribution patterns (Fartmann & Hermann, 2006), i.e., species that do not occur in areas with Atlantic and Mediterranean climates, but are widely distributed from continental Europe throughout temperate Asia. One of these continental species that are supposed to suffer from climate change is the woodland ringlet butterfly, *Erebia medusa* Fabricius (Lepidoptera: Nymphalidae) (Settele et al., 2008). *Erebia medusa* has already declined in several countries at the western (Atlantic) range margin, e.g., Belgium, Germany, France, and Luxembourg (van Swaay & Warren, 1999). In contrast, comparable population collapses have not

been observed in more continental countries. As *E. medusa*'s range mainly covers regions which are characterized by a continental climate with warm summers and cold winters (Kudrna et al., 2011) and as the observed population declines mostly occurred in the lowlands with warmer winters (Fartmann & Hermann, 2006), we considered rising winter temperatures to be the most likely factor by which climate change could drive the species' decline.

In order to test this hypothesis, we conducted a laboratory experiment where we exposed larvae of *E. medusa* to three winter temperature regimes reflecting (1) a warm winter characteristic of an area south-west of the current Atlantic range margin in the lowlands of south-western France; (2) a moderate winter typical of a foothill zone in central Germany, where population declines of *E. medusa* have already been observed; and (3) a cold winter that is characteristic of a montane zone in south-east Germany, where the species is common and populations are still stable. We studied the direct impact of the three temperature regimes on the larvae as well as the after-effects of these treatments on post-diapause larvae, pupae, and adults. In particular, we addressed the following questions. (1) Do elevated winter temperatures affect survival rates, body mass, and size of the immature and adult stages of *E. medusa*? (2) Do differences in rearing temperature during the winter influence the phenological development of larvae, pupae, and adults? And based on the results of our experiment, (3) what could be the long-term effects of warming winters on *E. medusa* populations?

Materials and methods

Study species

The woodland ringlet occurs widely from central and south-eastern Europe eastwards to East Asia. Its altitudinal range extends from the lowlands to the subalpine level (Ebert & Rennwald, 1991; Settele et al., 2008). *Erebia medusa* is on the wing for about 4 weeks between the start of May and mid-July (Ebert & Rennwald, 1991; Sonderegger, 2005). The time of adult emergence varies with altitude. Usually *E. medusa* is univoltine; however, at high elevations, it has a biennial life cycle (Sonderegger, 2005). The preferred habitats of *E. medusa* are nutrient-poor grasslands and woodland glades with a low land-use intensity (Ebert & Rennwald, 1991; Schraml & Fartmann, 2013). Various grass species are believed to serve as larval host plants; however, only few of them (viz., *Festuca ovina* L. aggregate, *Festuca rubra* L. aggregate) have been verified to be systematically used for oviposition (Fartmann, 2004). *Erebia medusa* overwinters in the third instar on the basis of grass tussocks or in the litter layer. After

hibernation, the larvae moult once more (Sonderegger, 2005; G. Stuhldreher, pers. obs.).

For many regions in Germany, declines have been observed (Bolz & Geyer, 2003; Fartmann, 2004; Reinhardt et al., 2007; Schulte et al., 2007). However, populations at low altitudes have disproportionally often become extinct compared to mountain populations (Fartmann & Hermann, 2006). Currently *E. medusa* is regarded as 'near threatened' in Germany (Reinhardt & Bolz, 2011).

Rearing of the species

In order to obtain eggs of *E. medusa*, 17 females were caught in five populations in the Upper Diemel Valley [central Germany, 51°23′N, 8°40′E, ca. 450 m above sea level (a.s.l.)] in June 2010. For 2 days, the females were kept individually in small cages with tussocks of *F. ovina*. In total, the females laid 263 eggs which they attached partly to the host plants, and partly to the gauze of the cage sides. The eggs were removed from the cages without detaching them from their substrates and stored outdoors until the larvae hatched. In addition to the eggs laid by the captured females, we collected 51 eggs in the habitat patches in which the females had been caught. From this total of 314 eggs, 265 larvae hatched. The newly hatched larvae were transferred onto *F. ovina* tussocks which had been planted in plastic boxes with net lids. Due to the lack of space in the climate cabinets (see below), rearing of each of the larvae in a separate box was not feasible. Instead, we used 15 boxes that received 16–18 larvae each. Therefore, it was not possible to follow the development of single individuals. Sibling larvae and offspring from females that belonged to the same population were allotted to the 15 rearing boxes as evenly as possible to minimize family and population effects. The *F. ovina* tussocks and the topsoil material in which they were planted were collected in nutrient-poor dry grasslands in the Upper Diemel Valley. The rearing boxes were stored outdoors in the Upper Diemel Valley and sheltered from rain until they were transferred to the climate cabinets. Soil moisture was kept similar in all of the boxes by watering the *F. ovina* plants by hand. After the end of the temperature treatments in the climate cabinets in mid-March, the larvae were again reared outdoors (as described above) until the adults emerged. After the end of the experiment, all butterflies were released into their native habitats.

Temperature treatments

We used climate cabinets to simulate three winter temperature regimes. One treatment, hereafter called 'moderate', was designed to reflect the average temperatures of the Diemel Valley in central Germany (ca. 300 m a.s.l.), a foothill zone of the Süderbergland mountain range where

E. medusa is still present, but has suffered from local extinctions in the lowest sites (Fartmann, 2004). The other two treatments, 'cold' and 'warm', had considerably lower and higher temperatures, respectively. In the cold treatment, we simulated the average winter temperatures of the montane zone of the Erz mountain range in south-east Germany (>500 m a.s.l.) where *E. medusa* is currently a common species and populations are stable (Reinhardt et al., 2007). In the warm treatment, we simulated the average winter temperatures of the department Landes in south-western France (ca. 100 m a.s.l.), a region situated south-west of *E. medusa*'s European range boundary (cf. Kudrna et al., 2011). The reference values of our treatments were long-term monthly mean air temperatures which we derived from grid datasets of Germany's National Meteorological Service (Deutscher Wetterdienst, pers. comm.) and from weather stations (Bordeaux, Dax, and Mont de Marsan) of the French National Meteorological Service (pers. comm.).

In total, five rearing boxes were assigned to each treatment. Each treatment was realized simultaneously in three 2000-W Kryothermostat climate cabinets (Ehret, Emmendingen, Germany), with chamber size 60 × 60 × 50 cm, so that each of the cabinets used for the same treatment contained one or two rearing boxes. In order to avoid possible effects of the cabinets, the boxes were placed in another cabinet belonging to the same treatment every 2 weeks. Each cabinet was equipped with four 20-W lamps emitting light of a spectral composition similar to that of outdoor daylight. The temperature treatments started on 12 November 2010 and lasted 4 months, until 14 March 2011. The temperature inside the climate cabinets was adjusted from month to month according to the average monthly means of the three reference regions (see above). In between, the temperature was kept constant without diurnal variation. The day-night-rhythm of the illumination was adjusted weekly according to the day length in the Diemel Valley (e.g., 9.0 h in mid-November, 7.9 h at winter solstice, and 11.7 h in mid-March). Actual temperature and relative humidity in the climate cabinets were recorded hourly by automatic data loggers (MicroLog; Fourier Systems, Rosh Ha'Ayin, Israel). The monthly means of these measurements are shown in Table 1, along with long-term (1961–1990) reference values of the monthly mean air temperature at a weather station of Germany's National Meteorological Service in Brilon near the Upper Diemel Valley (ca. 7 km west of the study area, at 472 m a.s.l.).

Body mass and size

All weight parameters were ascertained using a microbalance with an accuracy of 0.1 mg. At the beginning of

Table 1 Mean (± SE) temperature (T) and relative humidity (r.h.) in the climate cabinets during the rearing experiments as well as long-term (1961–1990) reference values of the mean air temperature at a weather station of Germany's National Meteorological Service in Brilon near the Upper Diemel Valley (ca. 7 km west of the study area at 472 m a.s.l.)

| | Treatment | | | | | | Weather station Brilon |
| | Warm | | Moderate | | Cold | | |
	T (°C)	r.h. (%)	T (°C)	r.h. (%)	T (°C)	r.h. (%)	T (°C)
November (second half)	7.1 ± 0.0	48.1 ± 0.1	3.9 ± 0.0	58.0 ± 0.1	1.8 ± 0.0	56.7 ± 0.1	2.4
December	6.6 ± 0.0	50.1 ± 0.1	1.2 ± 0.0	56.1 ± 0.2	−1.3 ± 0.0	55.1 ± 0.1	0.6
January	6.4 ± 0.0	50.0 ± 0.1	0.0 ± 0.0	52.8 ± 0.1	−2.4 ± 0.0	55.2 ± 0.1	−0.5
February	7.2 ± 0.0	50.2 ± 0.1	0.4 ± 0.0	52.7 ± 0.2	−2.1 ± 0.0	56.5 ± 0.1	0.0
March (first half)	9.2 ± 0.0	49.7 ± 0.1	3.4 ± 0.0	51.5 ± 0.2	1.0 ± 0.0	58.1 ± 0.2	1.4
Mean	7.0 ± 0.0	49.7 ± 0.0	1.4 ± 0.0	54.3 ± 0.1	−1.0 ± 0.0	56.0 ± 0.0	0.4

November 2010, each of the 179 larvae that were still alive and had reached the third instar was weighed. At the start of the temperature treatments, larval weight was equal (Figure 1A). At the end of the temperature treatments in mid-March, all larvae that had survived were counted and weighed again before being removed from the climate cabinets. Pupae were weighed 7 days after pupation and adults on the day of hatching after having excreted meconium. Forewing length, as an indicator of body size, was measured with a slide gauge (accuracy 0.05 mm) by confining the adults in a transparent cup with a flat bottom and fixating them temporarily with a piece of foam.

Statistical analysis

Differences in the survival rates among the three treatments were analysed at three stages of the experiment: at the end of the temperature treatments in mid-March, after pupation, and after adult eclosion. In each case, the status of the individuals (alive vs. dead) was set up as binary response variable in a binomial generalized linear mixed-effects model (GLMM). We used a logit link function and the Laplace approximation for parameter estimation. The type of treatment applied during the diapause in winter served as categorical predictor variable with three levels ('cold', 'moderate', and 'warm'). The cold treatment was always used as the reference level to which the two other levels of the predictor were compared using Wald tests. The identity of the box in which an individual was reared was set up as random factor. Phenology of adult eclosion was modelled in a similar way, but in this case two Poisson-GLMMs with a log link function and hatching date (number of days after 1 May 2011) as the response variable were fitted separately for males and females.

Differences in morphological parameters between groups of larvae, pupae, and adults were analysed by ANOVA if data were normally distributed with equal variances; otherwise, Kruskal–Wallis tests were applied. Pair-wise comparisons following ANOVA and Kruskal–Wallis tests were performed using the Holm–Sidak test and Dunn's test, respectively.

The GLMM analyses were conducted with R 3.0.1 (R Development Core Team, 2013) and the package nlme4 (Bates et al., 2014). The statistical tests were performed in SigmaPlot 11.0 (Systat Software, 2008).

Results

Direct effects of winter temperatures on the larvae

Rearing the larvae of *E. medusa* under different winter temperatures greatly influenced larval activity during the second half of the experiment. At the start of February, about half of the larvae and at the start of March nearly all of the larvae that were reared in the warm treatment had left their hibernation place in the litter layer and at the bottom of the grass tussocks and had resumed feeding. In contrast, most of the larvae in the two cooler treatments only resumed feeding in the second half of March after the end of the treatment. Consequently, larvae which had received the warm treatment had achieved a significantly higher body weight by the end of the experiment in mid-March than larvae from the cooler treatments (Figure 1B).

The warm winter treatment accelerated larval development. At the end of the temperature manipulations, 34% of the larvae reared under warm conditions had already reached the fourth instar, while all others were third instar. However, survival rates during the winter months were significantly lower in the warm treatment compared to the cold treatment (Tables 2 and 3).

After-effects of winter temperatures

The three winter temperature treatments did not affect mortality after hibernation, i.e., the survival rates of fully
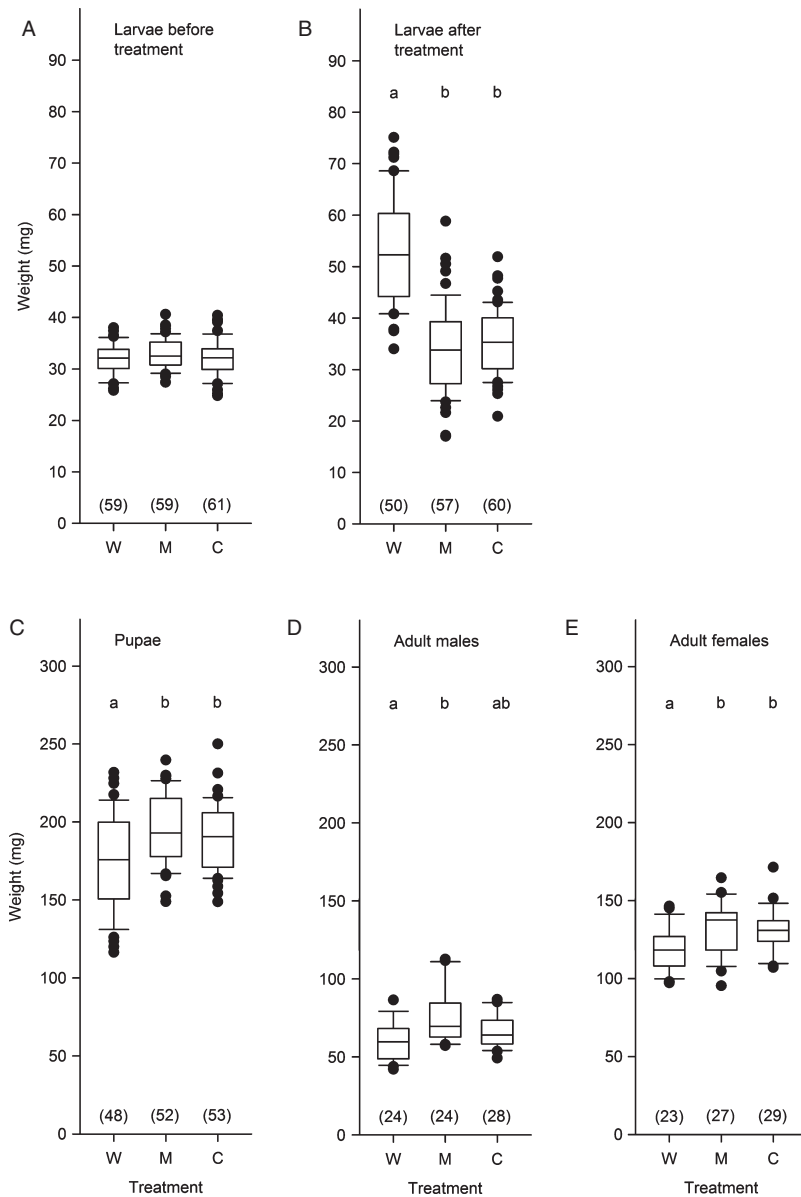
Figure 1 Body weights of larvae, pupae, and adults of *Erebia medusa*. (A) Larvae directly before the start of the temperature treatments (W, warm; M, moderate; C, cold), (B) larvae after exposure to various winter temperatures, (C) pupae, (D) adult males, and (E) adult females. The boxes indicate the median and 1st and 3rd quartile, the whiskers indicate 10th and 90th percentiles, and the dots are outliers. Sample sizes are indicated by numbers in parentheses below the boxes. Differences among treatments were analysed by ANOVA (larvae before treatment: $F_{2,173} = 1.46$, P = 0.24) or Kruskal–Wallis tests (larvae after treatment: H = 79.68, P<0.001; pupae: H = 12.74, P<0.01; adult males: H = 12.18, P<0.01; adult females: H = 11.93, P<0.01; all d.f. = 2). Different letters above the boxes within a panel indicate significant differences between treatments (Dunn's test for pair-wise multiple comparisons: P<0.05).

grown larvae and pupae did not differ (Tables 2 and 3). The same was true for pupal time and female forewing length (Table 4). In contrast, temperatures during hibernation had a significant influence on pupal and adult female weight, hatching time of the adults, and wing length of males (Figures 1C, E and 2, Table 4). Although the larvae from the warm winter treatment were much heavier than those from the two other treatments at the end of the experiment (Figure 1B), the reverse was true for the pupae (Figure 1C) and adult females (Figure 1E). Adult males that developed from larvae reared at high temperatures had significantly shorter forewings than those of the two other treatments (Table 4). The accelerated growth of the

larvae from the warm treatment resulted in the earlier emergence of adult males and females compared to adults that developed from larvae reared under the other two treatments (Figure 2, Table 5).

Discussion

Larval development

Our findings of an earlier onset of larval activity in the warm treatment is in accordance with the fact that, in many temperate insects, the period in which diapause is irreversible (i.e., when it cannot be terminated by external factors such as rising temperature) already ends in

Table 2 Absolute numbers and percentages of immature stages of *Erebia medusa* that survived after exposure to various temperatures during diapause in winter. Per box = % survival per rearing box; total = overall % survival (based on five rearing boxes per treatment)

Treatment	Pre-diapause larvae (n)	Survival during diapause (%)		Post-diapause larvae (n)	Survival after diapause (%)		Pupae (n)	Survival during pupal stage (%)		Adults (n)
		Per box	Total		Per box	Total		Per box	Total	
Cold	61	89, 100, 100, 100, 100	98.4	60	100, 100, 100, 100, 100	100.0	60	80, 100, 100, 100, 100	95.0	57
Moderate	59	92, 93, 100, 100, 100	96.6	57	86, 91, 92, 92, 100	93.0	53	83, 100, 100, 100, 100	96.2	51
Warm	59	77, 88, 92, 100, 100	86.4	51	100, 100, 100, 100, 100	100.0	51	67, 85, 92, 100, 100	92.2	47

Table 3 Results of the Generalized Linear Mixed Model analyses of the effect of temperature treatment applied during diapause in winter (categorical predictor variable with three levels, 'cold', 'moderate', and 'warm') on survival of immature stages of *Erebia medusa* (binary response variable). In each of the three analyses, the cold treatment was used as the reference level to which the two other levels of the predictor were compared using Wald tests. The identity of the box in which an individual was reared was set up as random factor

	Treatment	Estimate	SE	P
Diapausing larvae $n_{total} = 179$	Intercept	4.094	1.008	<0.001
	Moderate (M)	−0.744	1.239	ns
	Warm (W)	−2.242	1.078	<0.05
Post-diapause larvae $n_{total} = 168$	Intercept	3.799E+01	1.292E+07	ns
	M	−3.541E+01	1.292E+07	ns
	W	3.109E+02	1.597E+07	ns
Pupae $n_{total} = 164$	Intercept	3.698	0.947	<0.001
	M	0.187	1.409	ns
	W	−0.930	1.238	ns

ns = not significant.

mid-winter, long before the return of favourable conditions in spring (Hodek, 1996; Kostal, 2006). After diapause has ended, the insects remain in post-diapause quiescence until outdoor temperatures have increased above the lower threshold for development. As this study shows, constant temperatures around 7 °C are sufficient to allow for feeding and growth of larvae of *E. medusa* so that there is no need for the larvae in the warm treatment to remain in quiescence after the end of diapause. The thermal threshold for the end of the dormant phase of *E. medusa* larvae seems to be relatively low compared to those of other
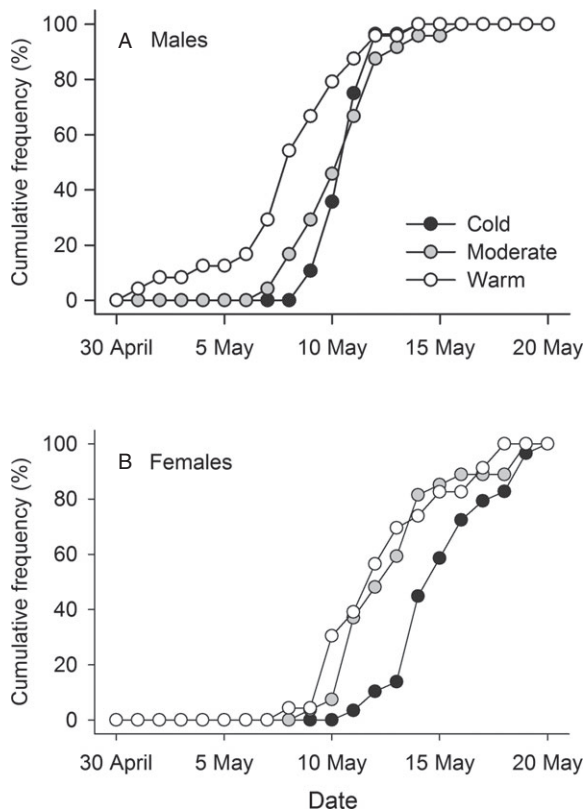
butterfly species such as the bay checkerspot [(*Euphydryas editha bayensis* (Sternitsky); Murphy & Weiss, 1988; Weiss et al., 1987] or the burnet moth *Zygaena trifolii* Esper (Wipking, 1995), which require temperatures of at least 12–15 and 16 °C, respectively. This can be seen as an adaption to a short growing season, in which growth and reproduction have to be completed within a short time-frame. In habitats that impose such a high degree of time-stress on organisms, resuming development as soon as environmental conditions have become favourable again in spring constitutes a selective advantage (Ingrisch, 1985). The ability to feed at low temperatures permitted the larvae in the warm treatment to compensate for the presumably increased metabolic rates and to continue their development earlier than the larvae reared at lower temperatures. This explains why the larvae in the warm winter treatment had a higher body weight at the time when the temperature treatments ended. Hence, we believe that elevated winter temperatures do not necessarily lead to a depletion of the energy reserves of the larvae—at least not in a constant environment, as in our experiment.

Although the larvae in the warm treatment had a considerable developmental edge at the end of the temperature manipulations in mid-March, the average date of adult emergence advanced by only 3 days compared to the coldest treatment. Given the fact that the larvae in the warm treatment ended diapause several weeks earlier than the larvae in the other two treatments, we conclude that post-diapause development rates were lower in the warm treatment. It seems unlikely that day length was the factor which controlled growth rates in spring, as photoperiod does not generally affect post-diapause morphogenesis (Tauber & Tauber, 1976). We believe that the different development rates were instead a reaction to the length of the period during which the larvae experienced very low temperatures, as has been demonstrated for some Orthoptera (Masaki, 1960; Ingrisch, 1985), the dragonfly *Aeschna mixta* Latreille (Schaller, 1968), and *Z. trifolii*

Table 4 Mean (± SE) duration of the pupal stage and forewing length of *Erebia medusa* individuals which developed from larvae that had been exposed to various winter temperatures (warm, moderate, and cold)

	Warm	Moderate	Cold	P
Pupal time (days)	19.6 ± 0.2 (45)	19.3 ± 0.2 (51)	19.3 ± 0.2 (53)	ns
Forewing length (mm) ♂♂	20.2 ± 0.2a (16)	21.9 ± 0.2b (18)	22.0 ± 0.1b (25)	<0.001
♀♀	21.3 ± 0.2 (22)	21.9 ± 0.2 (26)	21.7 ± 0.1 (29)	ns

Means within a row followed by different letters are significantly different (Dunn's test for pair-wise comparisons: P<0.05). Sample sizes are given in parentheses. Differences among treatments were analysed by Kruskal–Wallis tests; ns = not significant.

Figure 2 Phenology of adult eclosion, shown as cumulative frequencies of emerged (A) males and (B) females of *Erebia medusa* per treatment. The significance of the relationship between hatching day and treatment was tested using Poisson GLMMs (cf. Table 5).

Table 5 Results of the Generalized Linear Mixed Model analyses of the effect of temperature treatment applied during diapause in winter (categorical predictor variable with three levels, 'cold', 'moderate', and 'warm') on hatching date (number of days after 1 May 2011) of male and female adults of *Erebia medusa*. In both analyses, the cold treatment was used as the reference level to which the two other levels of the predictor were compared using Wald tests. The identity of the box in which an individual was reared was set up as random factor

	Treatment	Estimate	SE	P
Male adults	Intercept	2.288	0.060	<0.001
$n_{total} = 76$	Moderate (M)	−0.020	0.089	ns
	Warm (W)	−0.301	0.097	<0.01
Female adults	Intercept	1.989	0.115	<0.001
$n_{total} = 79$	M	−0.387	0.173	<0.05
	W	−0.497	0.181	<0.01

ns = not significant.

Body mass and size

At the time of pupation, larvae reared in the moderate and cold treatments had not only almost caught up on their developmental delay but weighed even more than larvae from the warm winter simulation. The higher body mass of pupae from the moderate and cold treatment resulted in adults that either had significantly longer forewings (males) or were heavier (females) than larvae reared in the warm treatment. These results seem to underpin the so-called temperature–size rule which refers to the negative relationship between developmental temperatures and adult size within a species in many ectothermic animals (Atkinson, 1994; Fischer & Karl, 2010). In this context, the compound interest hypothesis (Fischer & Fiedler, 2002) makes more nuanced predictions as a function of the species' voltinism. According to this hypothesis, the effects of low developmental temperatures should be stronger in multi- than in monovoltine species. Multivoltine species presumably gain high compound interest benefits from producing more generations per year, at the expense of being smaller in the adult stage. For obligatory monovoltine species, on the contrary, there is nothing to gain from

(Wipking, 1995). The larval stages of these species are thought to have evolved mechanisms for measuring the length of the chilling period. Unusually, short cold periods lead to slow post-diapause development so that ontogenesis is not completed too early in the year. Particularly long cold periods, on the contrary, speed up post-diapause development as they indicate that there is less time left to complete the life cycle than in normal years (Ingrisch, 1985).

shortening the pre-adult phase as they are not able to produce a second generation. Thus, the compound interest hypothesis predicts that differences in developmental temperatures in monovoltine species will not or will only slightly affect adult size and that temperature effects will be equally strong in both sexes (Fischer & Fiedler, 2002; Fischer & Karl, 2010). In accordance with this prediction, we only observed a few significant differences in adult body size or mass and all these differences were small.

Survival rates

Larval survival rates of *E. medusa* differed significantly with respect to rearing temperature during the winter months. Low temperatures proved to be the most favourable for larval survival, which supports the hypothesis that warm winters negatively affect the larvae of *E. medusa* (Fartmann & Hermann, 2006). However, the observed differences were smaller than we had expected considering the large temperature differences between the three treatments. There are two possible interpretations for this: either larvae of *E. medusa* are not as sensitive to high winter temperatures as previously assumed, or the impact of warm hibernation conditions has been unnaturally small due to the specific features of the treatments that were applied. For several reasons we favour the second interpretation. Firstly, we applied constant temperatures without any daily or multi-day fluctuations. In natural environments, which are characterized by great thermal variability, an increase in the mean temperature leads to a disproportionate increase of the energetic demand compared to thermally stable environments (Ruel & Ayres, 1999; Williams et al., 2012). This is due to the non-linear relationship between temperature and metabolic rate of ectothermic organisms and a mathematical property of such non-linear functions known as Jensen's inequality (Ruel & Ayres, 1999). Common garden experiments with larvae of the propertius duskywing (*Erynnis propertius* Scudder & Burgess) indeed proved that a thermally variable environment is more energy-demanding than a stable one (Williams et al., 2012). Secondly, air humidity in the climate cabinets was unnaturally low. This may have prevented the proliferation of pathogens, especially fungi, in the warm treatment, which are known to thrive best in moist environments (Hall & Papierok, 1982; Willmer, 1982). However, to test the impacts of temperature fluctuations and more humid conditions on overwintering survival, further experimental research is needed.

Implications for the fate of *Erebia medusa* in the face of global warming

Our study clearly showed that high winter temperatures lead to a higher mortality of *E. medusa* larvae. However,

the temperature differences between our treatments were higher than the increases in winter temperatures which have actually occurred in central Europe (1901–2008: +0.91 °C (Deutscher Wetterdienst, 2009) and which are expected to occur (1961–1990 vs. 2051–2080: +2–3 °C (Deutscher Wetterdienst, 2012). Nonetheless, we expect that the predicted warmer winters will have negative effects on the long-term survival of *E. medusa*. In our experiment, winter temperatures were constant, not showing the great thermal variability of natural environments with its deleterious effects on animals' energy reserves. Fluctuations in temperature are not only a general phenomenon in the habitats of *E. medusa*, they are also likely to become even greater if global warming continues. In temperate regions, higher mean winter temperatures are predicted to be accompanied by a reduced snow cover (Bale & Hayward, 2010; Kreyling, 2010). A thinner and shorter-lasting snow cover in turn leads to an increase in the frequency of soil freeze-thaw events and to more extreme variations in near-surface temperature.

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