Artificial warming advances egg-laying and decreases larval size in the dung beetle *Aphodius erraticus* (Coleoptera: Scarabaeidae) in a Tibetan alpine meadow

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The Tibetan Plateau is one of the areas in the world most sensitive to global warming. Dung beetles are the most abundant decomposer species group responsible for cattle dung removal in the Tibetan alpine meadow, and are critical to nutrient cycling and primary production of the grazing system. This study evaluates the possible effects of global warming on early life-history traits of dung beetles. We established three warmed open-top chambers (OTCs, about 2.3 °C higher than the control) and three ambient OTCs, each containing nine fresh dung pats that were bagged with a nylon screen (0.2 mm in mesh size). Twenty adults of a dominant coprophagous beetle species, *Aphodius erraticus* (Coleoptera: Scarabaeidae) had been put into each dung bag at the beginning of the experiment and the timings of egg-laying and hatching, and egg and larval size were followed for 80 days. Artificial warming advanced egg-laying and hatching by 4.1 and 7.2 days, respectively; warming decreased egg and larval size by 22.1% and 33.4%, respectively. This short-term study demonstrates that early life-history traits of *A. erraticus* are sensitive to artificial warming, which suggests that global warming may also change life history traits of other organisms in detritus-based systems.

**Introduction**

Global warming is expected to affect the phenology, physiology and distribution of plants and animals worldwide (Walther *et al*. 2002), especially in high-elevation habitats of the world (Walker *et al*. 2001). The Tibetan Plateau is considered to be one of the areas in the world most sensitive to global warming. The annual mean temperature of this plateau increased by about 0.8 °C during the latter part of the 20th century (Liu & Chen 2000), and is predicted to increase by 3 to 5 °C by the end of the 21st century (Solomon *et al*. 2007). Global warming is known to affect many aspects of plant and animal life histories. For example, warming usually advances the reproductive phenology of both plants and animals (Walther *et al*. 2002). An analysis of data collected over a 23-year period showed that almost all British butterfly species have advanced their eclosion time (Sparks *et al*. 2001). Similarly,
migratory birds and amphibians worldwide laid their eggs at earlier dates in the 1990s as compared with those in the 1970s (Crick et al. 1997, Forchhammer et al. 1998, Dunn & Winkler 1999). As a consequence of earlier reproduction, offspring size may also be affected by an increase in temperature. For example, egg size is often reduced in ectotherms under warmer conditions (Blanckenhorn 2000, Fischer et al. 2003). Blanckenhorn (2000) reported that increased temperatures decreased the egg size of flies under both field and laboratory conditions. Similarly, Fischer et al. (2003) showed that female butterflies produced larger eggs at low temperatures. These findings suggest that early life history traits of phytophagous animals are sensitive to global warming. However, the response of decomposers from detritus-based food webs to global warming has not received much attention.

The lack of research on the response of decomposers to global warming is surprising because decomposers facilitate important ecological functions such as nutrient cycling, bioturbation and plant growth enhancement (reviewed by Nichols et al. 2008). In grazing systems, most terrestrial primary production is not consumed by herbivores (Cebrian 2004). Furthermore, very little of the biomass consumed by large herbivores e.g., in alpine pastures is assimilated (Chew 1974). For example, millions of cattle graze the Tibetan Plateau, resulting in high volumes of feces in the landscape. Dung density is on average about 3600 pats per ha and can reach 5900 pats per ha, occupying ca. 24% of the total grassland area in summer grazing pastures (Wu & Sun 2010). Dung beetles are the most important decomposer species group responsible for cattle dung removal in this area (Wu & Sun 2010) and in many other ecosystems (Losey & Vaughan 2006). Thus, knowledge about the potential effect of global warming on population dynamics of these beetles would be helpful for sustainable management of the alpine ecosystem in a warmer future.

In the present study, we examined the timings of egg-laying and hatching, egg size (egg length) and larval size (body weight) of a coprophagous beetle species, Aphodius erraticus (Coleoptera: Scarabaeidae) in both warmed and ambient open-top chambers (OTCs) in an alpine meadow in the eastern part of the Tibetan Plateau, China. The specific objectives were to test (1) whether artificial warming advances the timings of egg-laying and hatching, and (2) whether warming decreases egg and larval sizes of the dung beetle.

**Material and methods**

**Study site and the beetle species**

This study was conducted in an alpine meadow in the eastern part of the Tibetan Plateau, China. The study area was geologically formed by tectonic movement that uplifted the plateau in the Cenozoic. Altitude ranges from 3500 to 3800 m. Climate is of the continental cold type, and is characterized by short and cool springs, summers, autumns and long winters. The annual mean temperature is between 0.6 °C and 1.1 °C, with the January minimum of –36 °C, and the July maximum of 26 °C. Annual mean precipitation, 80% of which occurs in May–August, is between 650 and 730 mm with considerable year-to-year fluctuations. The annual mean snow-cover time is 76 days.

The meadow where the study was conducted is mostly dominated by sedges such as Kobresia setchwanensis and Kobresia pygmaea, and grasses like Elymus dahuricus and Agrostis matsumurae. Forb species, including Saussurea nigrescens, Potentilla anserina, Aster alpine, Anemone trullifolia and Thalictrum alpinum are sometimes also abundant. The total vegetation coverage in mid-summer is over 80% and the average maximum plant height is about 30 cm. The decomposer community responsible for cattle dung removal is very diverse as in other areas (e.g., Mohr 1943). The two most abundant fly species are Scathophaga stercoraria and Calliphora vicina, while the coprophagous beetle community includes Aphodius erraticus, Aphodius rectus, Aphodius rusicola, Aphodius edgardi, Aphodius frater, Aphodius sp., Polydrusus sericeus, Canthon vigilans, Canthon sp., Germarostes aphodioides, Germarostes sp., Geotrupes egeriei, Bitoma sp. and Onthophagus schaefferi. Predaceous beetles include Philonthus rubripennis, Quedius (Microsaurus) liangshanensis and Sphaerites sp.
The dung beetle species *Aphodius erraticus* is a medium-sized (4.4–5.9 mm in body length) endocoprid that feeds and breeds within dung pats. This species is shiny black or dark brown and is moderately elongated (elytra much longer than pronotum) (Doube 1990). Its oviposition rate is low (Gittings & Giller 1997). An independent survey showed that *Aphodius erraticus* adults were active in dung pats from April to October, accounting for more than 70% of the total number of coprophagous beetles from June to September (Wu & Sun 2010).

The artificial warming experiment

In the year preceding our experiment, we fenced a 100 × 100 m plot where the vegetation cover was over 95%. Six open-top chambers (OTCs, 1 × 1 × 1 m) were randomly installed in the plot, with at least 3 m distance between them. The chamber sides were surrounded with thin (0.1 mm) steel screens (mesh size 0.2 × 0.2 mm), but half of the chambers were additionally covered with plastic screens with the sunlight transparency > 90%. All chambers were sunk into the soil to a depth of 20 cm.

Temperature was measured using data loggers (model DS1921G, Maxim Integrated Products, Sunnyvale, California, USA) that were placed in the center of each OTC. Over the course of the experiment, the daily mean temperature, which was calculated from the measurements made every 30 minutes over 24 hrs, was 12.1 °C in unwarmed chambers which was on average 2.3 °C less than in the warmed ones (Fig. 1A). The daily mean relative humidity (at 20 cm above ground level) was slightly higher in the ambient (89%) than in the warmed chambers (85.4%; Fig. 1B), which indicated that artificial warming did not change relative humidity much.

We collected adult beetles using 10 dung-baited traps for 2 days (30–31 May 2009) in the meadow, selecting only medium-sized (5.0–5.4 mm in body length) individuals for the experiment. This selection was made to avoid maternal effect on offspring size (e.g. Clutton-Brock et al. 1982, Mousseau & Dingle 1991). Because the beetle individuals were randomly trapped and selected, the sex ratio was assumed to be similar to that under natural conditions.

Dung was collected in the early morning (before 07:00) on 31 May 2009 from fresh droppings by yaks in a stall of a herd family. The dung was thoroughly mixed in a large bucket and then on the same day a standardized individual pat, about 400 g fresh weight, was added to a square-shaped bag (20 × 20 cm) made of nylon cloth (mesh size 0.2 mm). Subsequently, 20 adult beetles were added to each bag: this number reflected a beetle density in a nearby grazing pasture (ca. 40 individuals per 1000 g dung pat, see Wu et al. 2011). Finally, the bags were closed with a nylon cord.

The total number of dung bags was 54, 27 of which were placed in three warmed chambers and 27 in three unwarmed chambers. Each of the six chambers thus contained 9 bags evenly spaced within the chamber. During the course of the experiment, we removed six bags (one per chamber) nine times. The sampling dates were 4 June (dung age = 3 days), 7 June (6 d), 11 June (10 d), 16 June (15 d), 21 June (20 d), 26 June (25 d), 7 July (35 d), 21 July (50 d) and 20
August (80 d). On each sampling day, the eggs and larvae were removed by hand from each bag, counted and weighed; 40 g of the residual dung was sampled from each nylon bag and oven dried at 65 °C for 24 hrs to calculate the dung water content. Water content was similar (around 82%) during the first 15 days of the experiment, but was subsequently slightly lower in warmed (74% on average) than in unwarmed chambers (81%) (Fig. 1C).

Using a quadratic equation, the numbers of eggs and larvae were fitted against days since the start of the experiment. The timings of egg-laying and hatching were calculated by setting the egg or larval number to zero in the equations. The effect of artificial warming on egg size (egg length, mm) and larval size (dry body weight, mg) was determined using ANOVA with a linear mixed-effects model, in which the sampling date was set as a random factor and temperature as a fixed factor. These analyses were performed with the statistical package R (R Development Core Team 2011).

**Results**

**Timings of egg-laying and hatching**

The beetles began to lay eggs 6.1 days after the experiment started in the warmed chambers and after 10.2 days in the ambient OTCs (Fig. 2A). Warming advanced the timing of egg-laying by 4.1 days. Similarly, hatching occurred earlier in the warmed chambers (11.6 days after the experiment started) than in the ambient, control chambers (18.8 days) (Fig. 2B), i.e., warming advanced hatching time by 7.2 days.

**Egg and larval size**

Artificial warming reduced egg size greatly (by 22.1%) but at a marginally significant level ($F_{1,44} = 2.13, p = 0.076$). During the experiment, egg size in the warmed chambers was always smaller than in the unwarmed chambers (Fig. 3A). Likewise, the larvae were significantly ($F_{1,39} = 8.05, p < 0.05$) larger in the ambient chambers than in the warmed chambers (Fig. 3B). In the ambient chambers, larval size reached its maximum on the 35th day (32.7 mg per larva) and then declined gradually, whereas the larvae grew gradually with dung aging in the warmed chambers throughout the 80 day period. From the 50th to 80th day in the ambient OTCs, larval size showed a proportional decreased of < 1% per day.

**Discussion**

We have shown that artificial warming significantly changed the timings of egg laying and hatching in *Aphodius erraticus*, which is consistent with observations from previous studies on insect phenology in relation to global warming (Dewar & Watt 1992, reviewed by Bale *et al.* 2002, Liu *et al.* 2011). For example, a mid-sea-
son grasshopper, *Oedaleus asiaticus*, in northern China emerged 4.96 days earlier than normal when temperature increased by 1.5 °C (Guo et al. 2009). Roy and Sparks (2000) showed that in the United Kingdom a temperature increase of 1 °C advanced the first appearance of 25 out of 35 butterfly species by 2 to 10 days. Similarly, in central California, 16 out of 23 butterfly species advanced their first flight date by an average of 24 days over 31 years (Forister & Shapiro 2003, see also Stefanescu et al. 2003 for data on butterflies in Spain).

Advance of the timing of egg laying in *Aphodius erratius* may be directly attributed to an increase in temperature as demonstrated for other insect species (Bale et al. 2002). High temperature *per se* may result in cardiac stress in adults, such that to maintain other critical activities females have to lay eggs to reduce the allocation of energy for reproduction (Somero 2010). It is important to note that the pre-experimental status of adult females (e.g., size, age, mature or not, etc.) should not be the leading cause for differences in the timing of egg-laying in the beetle species. We intentionally selected adults of similar body size to avoid possible maternal effects (Moscardi et al. 1981), yet we cannot be sure of the age of the beetles or whether the females were gravid or not. Moreover, the beetles laid eggs more than 6 days after the experiment started even in the warmed chambers, but not at the very beginning. This suggests that the beetles might have had to assimilate some resources to accumulate energy before reproduction.

Advance of the timing of egg-hatching could not simply be attributed to earlier egg-laying in the warmed OTCs. The interval in the timing of egg hatching was 7.2 days, about 3 days more than the interval in the timing of egg laying (4.1 days). It seems that temperature increase *per se* must also have accelerated egg hatching. In addition, differences in relative humidity should not have affected the timing of egg laying by much. Adult beetles laid their eggs mostly at the dung-soil interface, which may cause egg hatching to be sensitive to changes in relative humidity. However, relative humidity was mostly above 80% in both warmed and unwarmed chambers, which should not be detrimental to beetle reproduction (Lumaret & Kirk 1987, Lazzari 1991).

For animals living in different habitats, egg size is often more stable than egg number (Lack 1954). However, we did not find significant variation in the number of eggs for this beetle (data not show), but observed a significant reduction in egg size under artificial warming. A possible reason for this is that early oviposition at high temperatures shortens the time of embryonic development (see Fox & Czesak 2000, Gillooly & Dodson 2000). Early egg-laying in the warmed OTCs implies that these beetles have less time to acquire resources before reproduction as compared with those in the ambient chambers. In addition, higher temperatures may decrease oocyte growth (Steigenga & Fischer 2007) and may increase costs of somatic maintenance of adults with a concomitantly lower proportion of resources available for egg production (Fox & Czesak 2000).
The smaller larvae in the warmed OTCs may directly be due to the smaller eggs, since small eggs cannot provide sufficient resources at least during the early-development stages of the larvae (Geister et al. 2009). However, the drier dung in the warmed chambers might be a more suitable food source because these beetle larvae are used to chew solid or fibrous material within drier dung pats (Halffter & Mathews 1966). Yet, drier dung seems not to overweight the negative effects of small eggs and high temperatures (on larval size), possibly because higher temperatures could induce cardiac stress, thus impairing the ability of these larvae to consume food (Somero 2010).

It is notable that larval size declined after reaching its peak in the ambient chambers. This is perhaps because a beetle larva often ceases feeding and clears its alimentary canal before pupation (Ludwig 1931). Interestingly, however, larval size increased gradually for a long period of time in the warmed chambers. There was a less than 1% difference in larval size over a period of 30 days from the 50th to 80th day in both the warmed and ambient chambers. This suggests that terminating the experiment on the 80th day was appropriate and covered the developmental period of the dung beetle larvae. Continuous growth of the warmed chamber larvae is partly because they have to reach a certain body size before pupation.

In conclusion, our results show that artificial warming may advance the timings of egg-laying and hatching of the dung beetle, which further result in smaller eggs and larvae. Although this study concentrated only on egg and larval stages, our results explicitly imply that global warming may have potentially important effects on the developmental stages of detritus-based organisms. More work should be done to fully explain the effects of global warming on other developmental stages of decomposers, particularly from sensitive alpine regions.

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