Running title: Contrasting warming effects on body size

Body size response to warming: time of the season matters in a tephritid fly

Xinqiang Xi, Xinwei Wu, Sören Nylin and Shucun Sun

*X. Xi, X. Wu and S. Sun (shcs@nju.edu.cn) The State Key Laboratory of Pharmaceutical
Biotechnology, College of Life Sciences, Nanjing University, 22 Hankou Road, Nanjing 210093,
China. -S. Sun also at Ecolab, Chengdu Institute of Biology, Chinese Academy of Sciences, No 9
Section 4 Renminnan Road, Chengdu 610041, China. -S. Nylin Department of Zoology,
Stockholm University, S-106 91 Stockholm, Sweden.*

Abstract

Whether shrinking body size is a universal response to climate change remains controversial. Moreover, the mechanisms underlying body size shifts are poorly understood. Here, assuming that life history traits evolve to maximize fitness according to life history plasticity theory, we hypothesized that under global warming temperate multivoltine insects should emerge earlier with a smaller body in the early growing season, but emerge later with a larger body in the late season. We tested this hypothesis by conducting two field artificial warming experiments in an alpine meadow: 1) with one pre-dispersal seed predator species (tephritid flies, *Tephritis femoralis*) and its two host plant species flowering in early and late growing seasons, respectively, and 2) with the tephritid flies and one host species with a flowering season that occupies parts of both the early and late growing seasons. These experiments were complemented by a microcosm chamber warming experiment, in which increasing and decreasing temperature trends were set to simulate temperature variation pattern in early and late growing seasons, respectively, but photoperiod was held constant. Warming generally advanced the adult emergence and decreased the body size (adult body mass) in the early season but delayed the emergence and increased the size in the late season in both field experiments, indicating that the seasonally different effects of warming on the fly body size was constant regardless of host plant identity. The chamber warming resulted in consistent responses of emerging timing and body size to the simulated seasonal warming, demonstrating that the temperature increase per se and its interaction with direction of temperature change, but not other correlated effects, should be primarily responsible for the observed contrasting shifts of body

34 size at different times of the season. Our results indicate that taking into account temperate
35 seasonal patterns of temperature variation could be of general importance for predicting animal
36 body size changes in the warmed future.

37 **Keywords:** global warming, rule of body size shrinking, tephritid flies, seed predator, host plant,
38 alpine meadow

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Introduction

Global average land surface temperature has increased by 0.85°C since the late 19th century, and it is predicted that the temperature will increase 4°C by 2100 unless appropriate measures are taken to slow down this warming rate (IPCC 2013). One of the most common responses to global warming is reduction of animal body size, which has been recorded in various taxa and even proposed as “the third universal response” alongside shifts in species distribution and biological phenology (Daufresne et al. 2009; Gardner et al. 2011), although the response is generally plastic rather than genetic (Gardner et al. 2011). Body size is in turn tightly related to almost every physiological and ecological trait of organisms (Peters 1983; Gillooly et al. 2001) and largely determines species interactions in biological communities (Woodward et al. 2005), which may further affect ecosystem structure and functioning (Petchey et al. 2008). Hence, it is not surprising that much attention has been paid to test the generality of this rule of body size reduction and to explore the mechanism underlying the body size response to environmental warming (Gardner et al. 2011; Bickford et al. 2011; Sebastian et al. 2012; Ohlberger 2013).

Recently, accumulated evidence shows an increasing number of exceptions to decreasing body size with increasing temperature – with many studies showing the opposite trend. These exceptions cannot be successfully explained by either Bergmann’s or James’s rule (James 1970) for endotherms or the “temperature-size rule” for ectotherms (Ray 1960; Atkinson 1994; Atkinson and Sibly 1997). Notably, these exceptions are almost exclusively found in temperate seasonal environments, such as common lizard (*Lacerta vivipara*) in European mountains (Chamaille-Jammes et al. 2006), tadpoles (*Rana kukunoris*) in Tibetan plateau (Zhao et al. 2014),

marten (*Martes americana*) in Alaska (Yom-Tov et al. 2008), eurasian otter (*Lutra lutra*) in Norway (Yom-Tov et al. 2006); mallard (*Anas platyrhynchos*) and teal (*Anas crecca*) in France (Guillemain et al. 2010), weddell seals (*Leptonychotes weddellii*) in Antarctic regions (Proffitt et al. 2007). Consistently, these exceptions are mostly accounted for by warming-induced increases in food quantity and quality (Yom-Tov et al. 2008) and growth efficiency (Walters and Hassall 2006) , as a result of temperature increase improving the seasonal physical environments (Moreno-Rueda and Rivas 2007, Gardner et al. 2011). These findings collectively suggest that seasonal environmental variation might be associated with the observed deviations from the proposed rule of body size responses to environmental warming (see also Atkinson 1994, Walters and Hassall 2006, Ohlberger 2013).

Temperate animals are largely constrained in growth and reproduction by low temperature in the very early and late growing seasons (Gotthard 2001), especially for ectotherms, whose body temperatures vary with environmental temperature and hence whose life–cycles are frequently strongly affected by seasonal thermal variation (Chown and Klok 2003, Van Dyck et al. 2015). A search for general patterns has to explore the responses of body size to warming in insects - the most diverse taxon in the world (Wilson 1999). Insects have evolved to possess a wide thermal tolerance but also fine-tuned phenotypic plasticity adapting their life cycles and life history traits to environmental changes (Nylin and Gotthard 1998). Theory assumes that insects in seasonal areas will have evolved to utilize the season favorable for growth and development in a way which maximizes their total fitness (Roff 1980, Abrams and Rowe 1996). For example, multivoltine species (i.e. those with a capacity for having more than one generation per year, a

substantial part of insects; Tauber and Tauber 1981) can be expected to take advantage of warming by increasing the number of generations in the growing season and/or increasing their survival rate in the following non-growing season (Roff 1980, Altermatt 2010). Under many theoretical scenarios there will be a fitness trade-off between short development times and large final sizes (Roff 1980, Abrams and Rowe 1996), where the former increases the chances of successfully achieving one or several complete generations whereas the latter often increases the chances of surviving to the next favorable season (Ohgushi 1996, Kovacs and Goodisman 2012) as well as generally providing a fecundity advantage (Nylin et al. 1980).

As multivoltine species should be adapted to maximize different goals at different times of the season, and since it is known that life history traits of insects can respond differently to temperature depending on the perceived date in the season (Gotthard et al. 2000), we here hypothesize that warming in early and late growing season should have different consequences on body size according to life history plasticity theory (Nylin and Gotthard 1998). Specifically, early emergence and an associated small body could be an evolutionary advantage in early season under global warming if the food resource is abundant, because it can be compensated by earlier reproduction, longer growth period for later generations, and possibly more generations (Atkinson 1994, Bryant et al. 1998). In contrast, late emergence and large bodies will be favored if warming occurs late in the season and hence permit a longer period of food consumption, because there is little potential for extra generations and because large bodies are often associated with high survival rate and high fecundity (Williams et al. 2015).

In order to test our hypothesis, we conducted experiments to examine the body size response to artificial warming in a host plant – parasitic seed predator system involving a tephritid fly species and three host plant species with different phenologies. We investigated the warming effect on timing of emergence and body size for the adult flies both early and late in the growing season in two field experiments, and in a complementary chamber experiment where temperatures were increasing or decreasing (mimicking time in the season) but photoperiod and moisture held constant. This was done to exclude confounding variables and to explore the possibility that, although insects generally respond to seasonal cues from photoperiod (Nylin and Gotthard 1998), a seed predator which is not exposed to direct sunlight could instead (or also) use the direction of change in temperature as cue. Our field experiments show that warming may have contrasting effects on organism body size depending on the timing of the warming and our chamber experiment suggests that the seasonal pattern in temperature variation could be an important indicator for predicting body size responses to warming in temperate animals.

Methods and materials

Study site

The field studies were conducted from early June to late September in an alpine meadow in Hongyuan Country, Sichuan province, China (32°48'N, 102°33'E). The meadow is located in eastern Tibetan Plateau with an altitude of 3500m above the sea level. According to the data collected at the Hongyuan County Climate Station (located 5 km from the study site) during 1961–2012, mean annual temperature is 0.9°C, with maximum monthly average temperature being 10.9°C (July) and minimum monthly average temperature -10.3°C (January); mean annual

precipitation is 744mm, which mainly occur during May to August (Zhao et al. 2014). Daily average temperature increases from early spring to summer and then drops from fall to winter, and the peak daily temperature often occurs at the end of July or the early August (Fig. S1). Meteorological data collected during the same time-period show that mean annual temperature increased at a rate of 0.29 °C per decade, with winter and summer temperature increases of 0.48 °C and 0.22 °C per decade (Mu et al. 2015).

The study meadow is typically dominated by sedges (*Kobresia setchwanensis*, *Blysmus sinocompressus*), grasses (*Agrostis hugoniana*, *Elymus nutans*, *Deschampsia caespitosa*), and forb species such as *Potentilla anserina*, *Saussurea nigrescens*, *Aster alpine*, *Anemone trullifolia* var. *linearis*, *Gentiana formosa*, *Euphrasia hirtella*, *Trollius farreri*, *Pedicularis* spp. and *Thalictrum alpinum*. Total vegetation coverage is more than 95% and plant height is ~30 cm (Wu et al. 2011). The meadow used in this study is used as a winter pasture, which is grazed by livestock during the winter only. Probably because of high plant species diversity (Xiang et al. 2009) and rich detritus resource (e.g. dung pats; Wu et al. 2011), the pollinator and parasitic species (e.g. Mu et al. 2014), as well as dung beetle species (Wu and Sun 2010), are diverse and abundant in the meadow.

The study species

A parasitic, pre-dispersal seed predator and three of its Asteraceae host plant species were included in this study. *Tephritis femoralis* Chen, 1938 (Diptera: Tephritidae) is a multivoltine tephritid fly, which usually completes two to three generations a year, depending on the phenological asynchrony with the host plants. Hibernation takes place in the adult stage. Field

measurements (by Xinqiang Xi) showed that body size of the adult fly ranged between 3.7 and 4.5mg for males and between 4.5 and 6.5mg for females, varying with the host plant and seasons. Adult flies of directly developing generations may mate and oviposit immediately after they emerge, and adult females oviposit in the capitula of different host plant species before flowering. Larval maggots grow up by feeding on immature seeds and possibly some parts of receptacles.

The host plant species that was chosen for the first generation of the fly (late May to mid-July; Fig. S1) was the early flowering Asteraceae species *Cremanthodium brunneopilosum*. Plant height of *C. brunneopilosum* is 52.19 ± 2.69 cm (N=30), and there are 4.51 ± 0.3 (N=30) capitula per plant and 170.6 ± 11.17 florets (N=30) per capitulum. Density of *C. brunneopilosum* ranges from between 0.3 and 0.7 m^{-2} in the meadow. The mid-season flowering Asteraceae species *Saussurea nigrescens* has a long flowering period (mid-June to late August; Fig. S1), and hence it is a dominant host species for the second generation flies and also serves as an important host species for the third ones. Plant height is 26.76 ± 0.96 cm (N=30). There are 3.35 ± 0.46 (N=30) capitula per plant and 38.2 ± 2.5 (N=30) florets per capitulum. The density of *S. nigrescens* is ca. 20 m^{-2} in the meadow. The late flowering Asteraceae species *Saussurea likiangensis* is the major host for the third generation. Its flowering period ranges between mid-August and mid-September (Fig. S1). There are 5 to 3 capitula per plant and 18.1 ± 0.45 (N=30) florets per capitulum. The density of *Saussurea likiangensis* is less than 1 m^{-2} in the meadow.

All the host species have a long fruiting period, such that the seed development time is often longer than the larval period of *T. femoralis*.

Field warming experiment

In order to test whether the flies differed in their body size response to warming between early and late seasons, we first conducted a field warming experiment involving two typical early and late seasonal flowering species (*C. brunneopilosum* and *S. likiangensis*, respectively) with their shared parasitic seed-predator *T. femoralis*. Four treatments were conducted, i.e. ambient and warmed *C. brunneopilosum* + *T. femoralis*, and ambient and warmed *S. likiangensis* + *T. femoralis*. The first two treatments were started in early season (5th June) and the latter two in the late season (8th August). Each treatment had 15 replicates.

Warming was artificially achieved using three infrared heaters (1600W, Heraeus Company, Germany), which were hung 1.3m high above the capitula of study plants; a similar dummy heater was also hung for the ambient treatment. The plants were arranged as a 2m*0.6m quadrat under each heater. Records from Hobo U23-003 temperature data logger (Onset Company, USA) showed that temperature (at 30 cm above ground surface) was enhanced by 2.15°C and 2.32 °C for early and late host plants, respectively, during the experiment.

In order to exclude the potential confounding effect of plant identity on the body size response to warming, we conducted a second field experiment involving the same fly species as in the first field experiment and a single host plant species (*S. nigrescens*), whose flowering season covers parts of both early and late seasons. As for the first experiment, there were four similar experimental treatments, one pair of treatments (ambient vs. warmed) were initiated in early season (25th June) and the other in the late season (8th August), with the same warming

facilities employed. Each treatment had 15 replicates. Mean temperature during the experiment was enhanced by 2.1°C and 2.47°C for the early and late season, respectively.

All the host plants used in the study were transplanted from the field at the end of September, 2013. More than 300 average-sized adult individuals of each plant species as well as the associated soil (12 cm and 10cm for diameter and depth respectively) were dug out and quickly moved to a similar sized plastic pot. From the transplanted plants, 30 healthy *C. brunneopilosum* and *S. likiangensis* as well 60 individuals (half used in early and another half in later season) of *S. nigrescens* were randomly chosen as hosts for *T. femoralis* maggots. We inserted one *T. femoralis* maggot (ca. 2mm in length) into one randomly chosen capitulum for a single host plant before starting the warming experiment. Subsequently, the capitula bearing maggots were encircled by mesh-sized nylon bags to prevent other flies from ovipositing and newly emerged flies from the developing capitula from escaping. For each species, fifteen plant individuals as well as the associated maggots were cultivated under the active heater, and the others were cultivated under the dummy heater in both field experiments. Host plants in the experiment were artificially pollinated and were irrigated regularly. Timing of emergence (the day after the beginning of the experiment) and sex (judged by the existence of ovipositor) was recorded for each newly emerged adult fly. Adult flies were put into a 10ml plastic centrifuge tube and then were placed in a 0°C refrigerator for two hours immediately after they emerged from the capitula, and subsequently the fresh mass were measured for each adult using an electronic balance.

The maggots used for injecting all the host plants in the experiment were derived from other plants of the same plant species. We first collected adult flies from field *Cremanthodium*

brunneopilosum, and then allowed them oviposit the *C. brunneopilosum* capitula in an injection box before flowering. The injection box was made from iron mesh supported by steel frame. Fifteen pairs of medium-sized female and male flies were released into the injection box to lay eggs into the capitulum of about 60 plants (transplanted from field) for three days, and then they were driven out of the box in order to ensure that maggots in the ensuing experiment had a similar birth day. We opened the capitulum carefully and collected 30 medium-sized maggots on the 9th day after the release of adult flies, and then these maggots could be used for the experiments.

Similarly, in order to obtain the maggots used for injecting *Saussurea nigrescens* and *Saussurea likiangensis* (for both early and late seasons), we collected pupae of the fly from *Cremanthodium brunneopilosum* and *Saussurea nigrescens* in the field (at different times corresponding to the experimental timing), respectively, and then reared them in an artificial cabinet. Temperatures in the cabinet were set as 23°C and 13°C for day and night, respectively, with a 16:8h light regime, and 75% moisture. Once the adult flies emerged, they were put into an injection box to allow them to oviposit into the capitula of *Saussurea nigrescens* or *Saussurea likiangensis*, and 9 days later we collected the maggots from the capitula and inserted them into the experimental capitula, following the same procedures as for the experiment with *Cremanthodium brunneopilosum*. The survival rate of the maggots (including both males and females) was above 90% in the experiments.

Chamber warming experiment

In order to exclude other confounding factors (e.g. photoperiod and relative humidity along the growing season) on the body size response to warming, we further conducted a chamber warming experiment, in which the maggots were cultivated in the artificial climate cabinets with strictly controlled environments. Similar to the field experiments, there were two factors, i.e. ambient vs. warmed, and increasing vs. decreasing temperature trends (to simulate early and late growing seasons, respectively) leading to four treatments in total, each treatment having 30 replicates. Initial temperatures were set at 13°C /4°C and 15°C /5°C for ambient and warmed treatments, respectively, for the two early season treatments. Both the day and the night temperatures were increased by 1°C every three days. For the two late growing season experiments, the initial cabinet temperatures were set at 23°C /11°C and 25°C /12°C for the ambient and warmed treatments, respectively. The daytime and nighttime temperatures were decreased by 1°C every three days. The temperature settings chosen were consistent with the temperature records from the local county weather station during the flowering season of *S. nigrescens* (Fig. S1). The light regime for all the cabinets was set as 16:8h, whereas the moisture was maintained at 75%.

We collected 120 maggots from the host plants of the second generation plants *S. nigrescens*, as we did the collection for *Saussurea likiangensis* in the second field experiment. Each maggot was inserted into one average-sized healthy *S. nigrescens* capitula that was collected from the field. The capitula and associated maggots were put into a glass bottle (diameter 10 cm and depth 10 cm), whose top was covered by a thin breathable paper in order to avoid extra water loss. In every three days, we carefully took out the maggots from the associated capitula and put them

into a fresh capitula that had been collected in field, in order to ensure that the quality of food resources was unaffected by capitulum dehydration. Timing of emergence and sex of each individual was recorded, and the fresh mass of adult flies was measured. The experiment was terminated when all flies had emerged from the capitula. Larval survival rate was above 85% for each treatment.

Data analysis

As male and female flies consistently differed in timing of emergence and terminal body size, two-way ANOVAs were employed to detect the effects of warming, sex and their interaction on the performance (timing of emergence and adult mass) of flies reared in the early season host *C. brunneopilosum* and the late season host *S. likiangensis*, respectively, in the first field experiment. Similarly, three-way ANOVAs were employed to detect the effect of warming, timing (early vs. late season), and sex, as well as their interactive effect, on the fly performance in the host plant *S. nigrescens* in the second field experiment and also in the chamber warming experiment. Once a significant effect was detected, Tukey HSD *post hoc* test were used to determine the difference among treatments. All the data analyses were conducted using R 3.1.0 (R Core Team 2014).

Results

When the maggots were placed into two different host plant species flowering at different times of the season, as in the first field experiment, warming significantly decreased the adult mass (by 0.6mg and 0.65mg for female and male flies, respectively) and advanced the timing of emergence of flies (by 4.59 and 6.29 days for female and male flies, respectively) reared in the early species *C. brunneopilosum* (Fig. 1, Table 1), but significantly increased the adult mass (by

1.1 and 0.71 mg for male and female, respectively) and postponed the timing of emergence (by 4.27 and 4.0 days for male and female, respectively) of flies reared in the late species *S. likiangensis* (Fig. 1, Table 1). The warming effect was consistent between the female and male flies in these two host plants.

In the second field experiment, when the maggots were reared in the same host plant species, *S. nigrescens*, but at different times in the season, warming showed similar effects on the timing of fly emergence and body mass, and regardless of sex (Fig. 2, Table 1), although the differences between some treatments were marginally significant ($P < 0.1$; Table S1).

In addition, when the maggots and the associated host capitula of *S. nigrescens* were cultivated in chamber, consistent trends were also observed: warming advanced the timing and reduced body size in the temperature-increasing treatments and vice versa for the temperature-decreasing treatments (except for the female emergence timing between the two early season treatments), independently of the fly sex (Fig. 3 Table 1, S1).

Discussion

We have clearly demonstrated a marked life history plasticity in animal body size in response to temperature, where the response of body size to warming differs in direction depending on the direction of change in base temperature - presumably signaling time of the season to the multivoltine tephritid fly studied. Different previous studies have documented either shrinking body size or increasing body size in response to warming (Gardner et al. 2011), but contrasting responses in body size has scarcely been explicitly documented in a single species (but see Hassall 2013).

If such contrasting responses are common in multivoltine insects, our finding suggests that exceptions to the rule of shrinking body size under global warming could be ubiquitous, and it also suggests that the non-significant/ neutral effect of warming on body size that revealed in previous studies could be simply due to the pooling of data collected in different seasons, as found in the present study (See Table 1). Moreover, provided that warming is predicted not to be consistent between seasons and between years, a warming effect on body size in one season or year could be cancelled out by that in another season or year and hence the overall response of body size to warming would not necessarily be directional (Williams et al 2015).

It is worthwhile to note that, in contrast to many of the previous similar studies, we employed a manipulative experimental strategy to determine the effect of warming on the terminal body size of the target species. In both types of warming experiment, we increased temperature only and other potential confounding factors were controlled. For example, host plants for different generations of *T. femoralis* were grown under similar conditions with similar subsequent managements. Previous studies often use temporal or spatial gradients to derive species responses of body size to global warming (Yom-Tov 2001, Gardner et al. 2014), acknowledging that they could be confounded by associated changes in precipitation and species interactions (Sheridan and Bickford 2011, Ohlberger 2013). Our study may avoid many of these confounding factors, with the caveat that the present study is short term and can detect only phenotypic plasticity in body size, not genetic changes.

Our results clearly indicate that the timing of artificial warming (early vs. later season) is the determining factor leading to the different sign of the warming effect on body size and timing of

emergence. The most contrasting seasonal environments in the first field experiment best shows the importance of timing of warming to the responses in a situation as close to the natural one as possible, since the maggots were grown in the earliest and latest flowering host species. The second field experiment - using the same host species for both cohorts - demonstrates that the contrasting effects were not due to host differences, and the chamber experiment rules out other confounding factors. Moreover, the variation in body size was significantly accounted for by the variation in the timing of emergence (Fig. S3). Accordingly, these contrasting warming effects are consistent with our hypothesis based on the life history plasticity theory (Nylin and Gotthard 1998).

Indeed, many previous empirical studies can be seen as indirectly supporting the core of our hypothesis: that body mass should often increase with warming immediately before an unfavorable season/ environment, but decrease with warming in an early favorable season/environment. For example, in potentially multivoltine insects it is a commonly observed pattern from split-brood experiments that individuals destined for diapause takes longer to develop than those destined for direct development (Nylin and Gotthard 1998), which will result in larger final mass in the former category unless growth rate is very plastic (Abrams and Rowe 1996). Although photoperiod is the main seasonal cue used to determine the developmental pathway, temperature also affects it, both directly and indirectly, by changing the seasonal occurrence of stages sensitive to photoperiod (Tauber and Tauber 1981). The differences in optimal life history between the two developmental pathways also translate to predicted geographical patterns in size/mass (Roff 1980). For instance, the body size of fall webworms

(*Hyphantria cunea*) increases with decreasing latitude (and thus increasing ambient temperature) from high latitude to 36° N, the area where only one generation occurs per year and thus all individuals must prepare for hibernation, but decreases at the latitude 36° N, where two generations occurs per year (Gomi and Takeda 1996). Such patterns are commonly observed in insects (e.g. Nygren et al. 2008, Nylin and Svärd 1991), demonstrating that ectotherms do not necessarily become smaller with increasing temperature when there are seasonal constraints (Williams et al 2015).

It should be noted, however, that such geographical patterns are the result of genetically based local adaptation interacting with plasticity in complex ways (Kivela et al. 2011, Nygren et al. 2008), where the generation time of the species in relation to the total growing season length is probably of major importance in determining which temperature-size pattern will prevail (Blanckenhorn and Demont 2004, Chown and Klok 2003, Kivela et al. 2011). Thus, ground crickets (*Palirhoeus* spp, *Canonopsis* spp, *Bothrometopus* spp, *Ectemnorhinus* spp) show increases in body size along an altitudinal gradient (and decreasing ambient temperatures) of a mountain in a non-seasonal environment where generations overlap, but decreases in body size along the altitudinal gradient of a mountain where there are strong seasonal constraints (Chown and Klok 2003).

Although larger bodies are not necessarily associated with greater lifetime fitness (e.g. due to size-dependent reproduction and competition), the core of our hypothesis can be further used to explain the positive effect of warming on body mass in more long-lived ectotherms. For example, the common lizards (*Lacerta vivip*) in European mountains show increased body size

when warming increases food availability before stepping into a cold winter (Chamaille-Jammes et al. 2006). *Rana ridibunda* and *R. lessonae* increased in body size during the period 1963–2003 in western Poland (Tryjanowski et al. 2006) presumably because warming increased insect abundance in the growing seasons (Gotthard 2001). Furthermore, our hypothesis is even consistent with the observation of increasing body mass under warming in endotherms. French mallard (*Anas platyrhynchos*) and teal (*Anas crecca*) have increased in body mass as a result of warming-induced increase in food availability before cold winters, when their body mass typically decline (Guillemain et al. 2010). Likewise, American marten (*Martes Americana*) increased in body mass when environmental warming lengthened the snow-free season before entering the food-scarce hibernation stage (Yom-Tov et al. 2008).

Our experiments have further demonstrated that the timing of warming *per se*, but not other correlated factors, can be enough to cause the observed contrasting effects. In the present study, food resource was not a limiting factor for maggot growth and reproduction in any of the experiments, as there were always unconsumed seeds in each capitulum at the end of the field experiments (data not shown) and capitula were regularly replaced with fresh ones in the chamber warming experiment. Moreover, photoperiod, one of the most important factors affecting insect growth and development rate because of its value as seasonal cue (Tauber and Tauber 1981, Nylin and Gotthard 1998), was held constant in the chamber experiment. This was done because maggots live within the capitula and might not be able to detect the light change, but, even if they are, our results demonstrate that cues from temperature alone are enough. Because temperature increase led to contrasting body size response to warming, our results

cannot be fully explained by the Bergmann's and James's rule (James 1970) or the temperature-size rule for ectotherms (Atkinson and Sibly 1997). One possible adaptive explanation is that the insects may have used the temperature variation pattern as a seasonal cue modulating their growth and development rates (Danks 2007). Continuously increasing temperature can be a reliable signal for maggots living within the capitula, indicating the transition from early to middle growing season, similar to the increasing photoperiod for some species as the signal for development transition (Nylin and Gotthard 1998, Gotthard 2001). In contrast, continuously decreasing temperature might be used as a signal indicating a transition from middle to late growth season. Thus, the contrasting body size responses to warming should be favored because the early emergence (and the small body which results if increased growth rate cannot compensate; Abrams and Rowe 1996) may allow for an extra generation (Bryant et al. 1998, Gotthard et al. 2000) and the late and large ones may ensure a high survival rate in the non-growing season as well as higher fecundity in the next growing season (Atkinson 1995, Kivela et al. 2011).

One possible physiological explanation for the observed patterns is an interaction of growth rate with ontogenetic changes in optimal temperatures (Ohlberger 2013). Because the optimal growth temperature often progressively increases with ontogenetic stage (Ohlberger 2013), we may speculate that increasing temperatures suppressed the maggot growth in the early season treatments but facilitated the growth in the late season treatments. Nevertheless, this speculation is not supported by the result of the growth rate (calculated as adult body mass divided by the timing of emergence), which was indistinguishable between warmed and ambient treatments in

all the three experiments (data not shown). Instead, the variation in body size was largely accounted for by the variation in timing of emergence (Fig. S3). Another possible explanation, which we cannot exclude, is that the initial temperature, but not the variation pattern, could be the cue for the contrasting responses. More studies are needed to test whether the initial temperature or the variation pattern and how they shaped animal growth and development.

In conclusion, we have shown that the multivoltine tephritid fly under study has contrasting responses to artificial warming in the early and late season, respectively, through a methodology involving both field and chamber experiments. Our finding suggests that numerous exceptions to the rule of shrinking body size under global warming may be explained from an evolutionary perspective by an extension of the life history plasticity theory. We suggest that temperate seasonal patterns of temperature variation could be taken a mechanism underlying diverse responses of body size to warming in both endotherms and ectotherms in future research.

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References

- Abrams, P. A. and Rowe, L. 1996. The Effects of Predation on the Age and Size of Maturity of Prey. - *Evolution* 50: 1052-1061.
- Altermatt, F. 2010. Climatic warming increases voltinism in European butterflies and moths. -

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- 415 Proc. R. Soc. B 277: 1281-1287.
- 416 Atkinson, D. 1994. Temperature and organism size—a biological law for ectotherms? – Adv. Eco.
417 Res. 25: 1-58.
- 418 Atkinson, D. 1995. Effects of temperature on the size of aquatic ectotherms: Exceptions to the
419 general rule. – J. Therm. Biol. 20: 61-74.
- 420 Atkinson, D. and Sibly, R. M. 1997. Why are organisms usually bigger in colder environments?
421 Making sense of a life history puzzle. - Trends Ecol. Evol. 12: 235-239.
- 422 Bickford, D. P., et al. 2011. Climate change responses: forgetting frogs, ferns and flies? - Trends
423 Ecol. Evol. 26: 553-554; author reply 555-556.
- 424 Blanckenhorn, W. and Demont, M. 2004. Bergmann and converse Bergmann latitudinal clines in
425 arthropods: two ends of a continuum? – Integr. Comp. Biol. 44: 413-424.
- 426 Bryant, S. R., et al. 1998. Modification of the triangle method of degree-day accumulation to
427 allow for behavioural thermoregulation in insects. - J. Appl. Ecol. 35: 921-927.
- 428 Chamaille-Jammes, S., et al. 2006. Global warming and positive fitness response in mountain
429 populations of common lizards *Lacerta vivipara*. - Global Change Biol. 12: 392-402.
- 430 Chown, S. L. and Klok, C. J. 2003. Altitudinal body size clines: latitudinal effects associated
431 with changing seasonality. - Ecography 26: 445-455.
- 432 Danks, H. 2007. The elements of seasonal adaptations in insects. - Can. Entomol. 139: 1-44.
- 433 Daufresne, M., et al. 2009. Global warming benefits the small in aquatic ecosystems. - Proc. Natl
434 Acad. Sci. USA 106: 12788-12793.
- 435 Gardner, J. L., et al. 2014. Dynamic size responses to climate change: prevailing effects of rising

-
- 436 temperature drive long-term body size increases in a semi-arid passerine. - *Global Change*
437 *Biol.* 20: 2062-2075.
- 438 Gardner, J. L., et al. 2011. Declining body size: a third universal response to warming? - *Trends*
439 *Ecol. Evol.* 26: 285-291.
- 440 Gillooly, J. F., et al. 2001. Effects of Size and Temperature on Metabolic Rate. - *Science* 293:
441 2248-2251.
- 442 Gomi, T. and Takeda, M. 1996. Changes in Life-History Traits in the Fall Webworm within Half
443 a Century of Introduction to Japan. - *Funct. Ecol.* 10: 384-389.
- 444 Gotthard, K. 2001. Growth strategies of ectothermic animals in temperate environments. *In* D.
445 Atkinson and M. Thorndyke (eds.), *Environment and animal development: Genes, life*
446 *histories, and plasticity*, pp. 287-303. BIOS Scientific Publishers, Oxford.
- 447 Gotthard, K., et al. 2000. Individual state controls temperature dependence in a butterfly
448 (*Lasiommata maera*). - *Proc. R. Soc. B* 267: 589-593.
- 449 Guillemain, M., et al. 2010. Wintering French mallard and teal are heavier and in better body
450 condition than 30 years ago: effects of a changing environment? - *Ambio* 39: 170-180.
- 451 IPCC. 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group*
452 *I to the Fifth Assessment Report of the Intergovernmental Panel on Climate*
453 *Change*.-Landon, Cambridge Univ. Press.
- 454 James, F. C. 1970. Geographic Size Variation in birds and its relationship to Climate. - *Ecology*
455 51: 365-390.
- 456 Kivela, S. M., et al. 2011. Latitudinal insect body size clines revisited: a critical evaluation of the

-
- 457 saw-tooth model. - J. Anim. Ecol. 80: 1184-1195.
- 458 Kovacs, J. L. and Goodisman, M. A. 2012. Effects of size, shape, genotype, and mating status on
459 queen overwintering survival in the social wasp *Vespula maculifrons*. - Environ. Entomol.
460 41: 1612-1620.
- 461 Moreno-Rueda, G. and Rivas, J. M. 2007. Recent changes in allometric relationships among
462 morphological traits in the dipper (*Cinclus cinclus*). – J. Orn. 148: 489-494.
- 463 Mu, J., et al. 2014. Domesticated honeybees evolutionarily reduce flower nectar volume in a
464 Tibetan lotus. - Ecology. 95. 3161-3172.
- 465 Mu, J., et al. 2015. Artificial asymmetric warming dramatically reduces nectar production in a
466 Tibetan lotus. Ann. Bot. Special issue on plant and climate. doi:10.1093/aob/mcv042.
- 467 Nygren, G. H., et al. 2008. Latitudinal body size clines in the butterfly *Polyommatus icarus* are
468 shaped by gene-environment interactions. - J. Ins. Science 1-13.
- 469 Nylin, S. and Gotthard, K. 1998. Plasticity in life-history traits. – Annu. Rev. Entomol. 43:
470 63-83.
- 471 Nylin, S. and Svärd, L. 1991. Latitudinal patterns in the size of European butterflies. - Ecography
472 14: 192-202.
- 473 Ohgushi, T. 1996. Consequences of adult size for survival and reproductive performance in a
474 herbivorous ladybird beetle. – Ecol. Entomol. 21: 47-55.
- 475 Ohlberger, J. 2013. Climate warming and ectotherm body size – from individual physiology to
476 community ecology. - Funct. Ecol. 27: 991-1001.
- 477 Petchey, O. L., et al. 2008. Size, foraging, and food web structure. - Proc. Natl Acad. Sci. USA

-
- 478 105: 4191-4196.
- 479 Peters, R. 1983. The ecological implications of body size. Cambridge Univ. Press.
- 480 Proffitt, K. M., et al. 2007. Exploring linkages between abiotic oceanographic processes and a
481 top-trophic predator in an Antarctic ecosystem. - *Ecosystems* 10: 120-127.
- 482 Ray, C. 1960. The application of Bergmann's and Allen's rules to the poikilotherms. - *J Morphol.*
483 106: 85-108.
- 484 Roff, D. 1980. Optimizing development time in a seasonal environment: The 'ups and downs' of
485 clinal variation. - *Oecologia* 45: 202-208.
- 486 Sebastian, P., et al. 2012. Effects of water temperature and mixed layer depth on zooplankton
487 body size. - *Mar. Biol.* 159: 2431-2440.
- 488 Sheridan, J. A. and Bickford, D. 2011. Shrinking body size as an ecological response to climate
489 change. - *Nature Clim. Change* 1: 401-406.
- 490 Tauber, C. A. and Tauber, M. J. 1981. Insect Seasonal Cycles: Genetics and Evolution. - *Annu.*
491 *Rev. Ecol. Syst.* 12: 281-308.
- 492 Tryjanowski, P., et al. 2006. Is body size of the water frog *Rana esculenta* complex responding to
493 climate change? - *Naturwissenschaften* 93: 110-113.
- 494 Van Dyck, H., et al. 2015. The lost generation hypothesis: could climate change drive ectotherms
495 into a developmental trap? - *Oikos* 124: 54-61.
- 496 Walters, R. J. and Hassall, M. 2006. The temperature - size rule in ectotherms: May a general
497 explanation exist after all? - *Am. Nat.* 167: 510-523.
- 498 Williams, C. M., et al. 2015. A cross-seasonal perspective on local adaptation: metabolic

-
- 499 plasticity mediates responses to winter in a thermal-generalist moth. - *Funct. Ecol.* 29:
500 549-561.
- 501 Williams, C. M., H. A. L. Henry, and B. J. Sinclair. 2015. Cold truths: how winter drives
502 responses of terrestrial organisms to climate change. *Biol. Rev.* 90:214-235.
- 503 Wilson, E. O. 1999. *The diversity of life.* - Norton, New York, USA.
- 504 Woodward, G., et al. 2005. Body size in ecological networks. - *Trends Ecol. Evol.* 20: 402-409.
- 505 Wu, X., et al. 2011. A brown-world cascade in the dung decomposer food web of an alpine
506 meadow: effects of predator interactions and warming. - *Ecol. Monogr.* 81: 313-328.
- 507 Wu, X. and Sun, S. 2010. The roles of beetles and flies in yak dung removal in an alpine meadow
508 of eastern Qinghai-Tibetan Plateau. - *Ecoscience* 17: 146-155.
- 509 Xiang, S., et al. 2009. Current status and future prospects of Zoige Marsh in Eastern
510 Qinghai-Tibet Plateau. - *Ecol. Engin.* 35: 553-562.
- 511 Yom-Tov, Y. 2001. Global warming and body mass decline in Israeli passerine birds. - *Proc. R.*
512 *Soc. B* 268: 947-952.
- 513 Yom-Tov, Y., et al. 2006. Body size changes among otters, *Lutra lutra*, in Norway: the possible
514 effects of food availability and global warming. - *Oecologia* 150: 155-160.
- 515 Yom-Tov, Y., et al. 2008. Recent increase in body size of the American marten *Martes americana*
516 in Alaska. - *Biol. J. Linn. Soc.* 93: 701-707.
- 517 Zhao, J., et al. 2014. Artificial warming increases size at metamorphosis in plateau frogs (*Rana*
518 *kukunoris*) in the presence of predators. - *Aquat. Ecol.* 48: 423-424.
- 519

Table 1. ANOVA results showing the effects of warming, sex and timing of warming on the timing of emergence and adult mass in all the field and chamber warming experiments.

	Timing of emergence			Adult mass		
	d.f.	F	P	d.f.	F	P
<i>Cremanthodium brunneopilosum</i>						
Warming	1	45.75	<0.001	1	7.15	0.013
Sex	1	35.98	<0.001	1	29.88	<0.001
Warming*Sex	1	0.99	0.33	1	0.16	0.69
Residuals	26			26		
<i>Saussurea likiangensis</i>						
Warming	1	8.02	0.009	1	13.68	0.010
Sex	1	2.50	0.13	1	9.79	0.0043
Warming*sex	1	0.38	0.54	1	0.62	0.44
Residuals	26			26		
<i>Saussurea nigrescens</i>						
Warming	1	0.54	0.47	1	0.36	0.55
Timing	1	110.73	<0.001	1	87.35	<0.001
Sex	1	26.58	<0.001	1	36.30	<0.001
Warming*Timing	1	38.69	<0.001	1	14.23	<0.001
Warming*Sex	1	0.16	0.69	1	0.26	0.61
Timing*Sex	1	0.78	0.38	1	0.029	0.87
Warming*Timing*Sex	1	1.07	0.34	1	0.19	0.66
Residuals	52			52		
Chamber warming experiment						
Warming	1	4.42	0.039	1	1.13	0.29
Timing	1	40.63	<0.001	1	16.45	<0.001
Sex	1	21.46	<0.001	1	32.69	<0.001
Warming*Timing	1	27.77	<0.001	1	15.84	<0.001
Warming*Sex	1	0.22	0.64	1	0.14	0.71
Timing*Sex	1	8.69	0.0042	1	4.63	0.034
Warming*Timing*Sex	1	0.43	0.51	1	0.39	0.53
Residuals	81			81		

Figure legends

Figure 1. Timing of emergence and adult mass in ambient and warmed treatments for both male and female flies grown in early season host *Cremanthodium brunneopilosum* and late season host *Saussurea likiangensis*. Ends of the boxes represent the 25th and 75th percentiles of the data, while ends of whiskers represent the minimum and maximum of the data. Line and black filled squares within the box represent the median and mean of the responses, respectively. Different letters above the boxes denote significant differences among treatments ($P < 0.05$), and the number under the boxes are the sample size.

Figure 2. Timing of emergence and adult mass in ambient and warmed treatments for both male and female flies grown in the host *Saussurea nigrescens* that flowered in both early and late seasons. The descriptions about the boxes and statistics are the same for Figure 1..

Figure 3. Timing of emergence and adult mass in ambient and warmed treatments for both male and female flies that were fed with fresh capitula of *Saussurea nigrescens* in chambers, in which temperature was set to simulate the early and late seasons. The descriptions about the boxes and statistics are the same for Figure 1.

Figure 1

Figure 2

Figure 3

Figure S1 Daily average temperatures during 1965 to 2013 and the flower phenology of the three host plant species in study meadow. The flowering phenology was determined following the method in Liu et al. (2012).

Liu, Yinzhan Mu, Junpeng, Niklas, Karl J, Li, Guoyong, Sun, Shucun. (2012). Global warming reduces plant reproductive output for temperate multi-inflorescence species on the Tibetan plateau. *New Phytologist* **195**: 427-436.

569 Figure S2. Temperature variation recorded for both ambient and warmed treatments during the
570 field experiment.

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Figure S3. Regression relationships between timing of emergence and adult mass for the flies in the field warming experiment. cb, *Cremanthodium brunneopilosum*; sl, *Saussurea likiangensis*; sne, *Saussurea nigrescens* in early season; snl, *Saussurea nigrescens* in late season. N=30, $R^2 > 0.58$ and $P < 0.001$ for all the host plants.

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Table S1 Results of TukeyHSD *post hoc* tests showing differences between treatments for timing of emergence and adult mass in the two field warming experiment and chamber warming experiment. FW, warmed females; FA, ambient females; MW, warmed males; MA, ambient males.

	Timing of emergence		Adult mass	
	Difference	P	Difference	P
<i>Cremanthodium brunneopilosum</i>				
FW-FA	-4.60	0.0018	-0.60	0.016
MA-FA	-4.22	0.0088	-1.12	<0.001
MW-FW	-5.91	<0.001	-1.16	<0.001
MW-MA	-6.29	<0.001	-0.64	0.028
<i>Saussurea likiangensis</i>				
FW-FA	4.26	0.018	1.09	0.0043
MA-FA	-1.71	0.68	-0.83	0.079
MW-FW	-1.38	0.77	-0.93	0.025
MW-MA	4.6	0.038	-0.98	0.040
<i>Saussurea nigrescens</i> in early season				
FW-FA	-3.28	0.066	-1.29	<0.001
MA-FA	-4.30	0.093	-0.36	0.0023
MW-FW	-4.39	0.061	0.14	0.14
MW-MA	-3.37	0.12	0.43	0.084
<i>Saussurea nigrescens</i> in late season				
FW-FA	2.75	0.071	1.09	0.010
MA-FA	-3.39	0.25	-0.69	0.38
MW-FW	-4.00	0.15	-1.20	0.016
MW-MA	2.14	0.87	0.57	0.75
Simulated early season				
FW-FA	-2.46	0.57	-1.179	0.0020
MA-FA	-5.05	0.0085	-1.49	<0.001
MW-FW	-5.51	0.0081	-1.01	0.0072
MW-MA	-2.92	0.32	-0.68	0.044
Simulated late season				
FW-FA	3.90	0.078	0.71	0.076
MA-FA	-1.87	0.84	-0.72	0.058
MW-FW	-0.54	0.99	-0.68	0.10
MW-MA	5.24	0.0031	0.75	0.043