

PART OF A SPECIAL ISSUE ON PLANTS AND CLIMATE CHANGE

Artificial asymmetric warming reduces nectar yield in a Tibetan alpine species of Asteraceae

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Received: 6 February 2015 Returned for revision: 12 February 2015 Accepted: 27 February 2015 Published electronically: 28 April 2015

● **Background and Aims** Asymmetric warming is one of the distinguishing features of global climate change, in which winter and night-time temperatures are predicted to increase more than summer and diurnal temperatures. Winter warming weakens vernalization and hence decreases the potential to flower for some perennial herbs, and night warming can reduce carbohydrate concentrations in storage organs. This study therefore hypothesized that asymmetric warming should act to reduce flower number and nectar production per flower in a perennial herb, *Saussurea nigrescens*, a key nectar plant for pollinators in Tibetan alpine meadows.

● **Methods** A long-term (6 years) warming experiment was conducted using open-top chambers placed in a natural meadow and manipulated to achieve asymmetric increases in temperature, as follows: a mean annual increase of 0.7 and 2.7 °C during the growing and non-growing seasons, respectively, combined with an increase of 1.6 and 2.8 °C in the daytime and night-time, respectively, from June to August. Measurements were taken of nectar volume and concentration (sucrose content), and also of leaf non-structural carbohydrate content and plant morphology.

● **Key Results** Six years of experimental warming resulted in reductions in nectar volume per floret (64.7 % of control), floret number per capitulum (8.7 %) and capitulum number per plant (32.5 %), whereas nectar concentration remained unchanged. Depletion of leaf non-structural carbohydrates was significantly higher in the warmed than in the ambient condition. Overall plant density was also reduced by warming, which, when combined with reductions in flower development and nectar volumes, led to a reduction of ~90 % in nectar production per unit area.

● **Conclusions** The negative effect of asymmetric warming on nectar yields in *S. nigrescens* may be explained by a concomitant depletion of leaf non-structural carbohydrates. The results thus highlight a novel aspect of how climate change might affect plant–pollinator interactions and plant reproduction via induction of allocation shifts for plants growing in communities subject to asymmetric warming.

Key words: Global climate change, asymmetric warming, nectar rewards, nectar yield, alpine meadow, *Saussurea nigrescens*, Asteraceae, plant–pollinator interactions, non-structural carbohydrates.

INTRODUCTION

By virtue of their close mutualistic association, pollinator population density and activity are largely dependent upon floral resource availability and quality (Hanley *et al.*, 2008, 2014; Brandenburg *et al.*, 2012; Scaven and Rafferty, 2013). For example, pollinators typically show fidelity towards plants producing more and higher quality nectar (Lake and Hughes, 1999; Mitchell *et al.*, 2004), such that nectar production directly affects pollinator activity (Klinkhamer and de Jong, 1990; Kudo and Harder, 2005) and pollinator community structure (Potts *et al.*, 2004). Similarly, any decrease in floral quantity and quality often leads to a reduction of pollinator abundance and activity (Larsson and Franzen, 2007; Wallisdevries *et al.*, 2012), which has the potential to disrupt mutualistic plant–pollinator relationships (Roulston and Goodell, 2011; Rafferty and Ives, 2012). Indeed, according to a USDA report (USDA, 2013), world-wide nectar production has declined yearly.

Although this phenomenon is often attributed to the decline of bee colonies and the disruption of plant–animal interactions due to climate change (Memmott *et al.*, 2007), it is not clear whether the decline in world-wide nectar production is also attributable to the decrease in nectar production resulting from global warming.

Indeed, many factors, including habitat loss, invasive species, pathogens and pesticide usage, currently threaten pollinator communities globally (Fortuna and Bascompte, 2006; Alston *et al.*, 2007; Cox-Foster *et al.*, 2007; Stout and Morales, 2009). The effect of climate change on plant–pollinator interactions is another factor, albeit one that remains relatively unknown. In recent decades, studies have shown that changes in precipitation, temperature, CO₂ concentration and nitrogen deposition can alter plant phenology, flower abundance and floral quality, which can further change flower attractiveness and nutritional rewards to pollinators and hence affect plant–pollinator interactions (Burkle and Alarcón, 2011; Hoover *et al.*, 2012).

For example, elevated temperature can increase floral nectar sugar concentration (Singh, 2013), and thus directly enhance the abundance and diversity of pollinators (Scaven and Rafferty, 2013; Singh, 2013). Similarly, high plant nectar yields often occur in years with high precipitation (Petanidou and Smets, 1996, 1999; Lloyd *et al.*, 2002), thereby providing greater rewards to pollinators (Hoover *et al.*, 2012). In contrast, few studies have focused on the effect of asymmetric warming on flowering potential and floral nectar production, which can significantly affect plant–pollinator mutualisms. Meteorological records and climate model projections have shown that temperatures have increased more in the winter than in the summer and that temperatures have increased more during the night-time than during the daytime, particularly at high latitudes and altitudes (Bonsal *et al.*, 2001; Shabbar and Bonsal, 2003; Solomon *et al.*, 2007; Xia *et al.*, 2014). Since many temperate plants require vernalization and must experience a period of low winter temperature to initiate flower production (Chouard, 1960), winter warming is likely to reduce flowering potential (Hennessy and Clayton-Greene, 1995; Cook *et al.*, 2012; Liu *et al.*, 2012). Indeed, warming disrupts vernalization and results in reduced flower and seed production (Saure, 1985; Hennessy and Clayton-Greene, 1995; Liu *et al.*, 2012) and delayed flowering (Warner and Erwin, 2006; Liu *et al.*, 2012). In addition, night-time warming may reduce nectar yield by increasing plant respiration and causing a draw-down on non-structural carbohydrate reserves (Wan *et al.*, 2009) that would otherwise be allocated to nectar production (Davis *et al.*, 1998). It is therefore surprising that comparatively few studies have examined whether asymmetric warming significantly affects nectar production (Scaven and Rafferty, 2013; but see Jakobsen and Kristjánsson, 1994).

The extensive alpine meadows of the Tibetan Plateau provide ideal sites to explore asymmetric warming effects on nectar production. The Tibetan Plateau has experienced significant warming over the past 50 years, the average air temperature having increased 0.21 °C per decade (Stocker *et al.*, 2013; You *et al.*, 2013), with likely significant implications for the ecology of the high-altitude alpine meadows that typify the region. The aim of this study was to simulate asymmetric warming and quantify its impact on nectar yields of an ecologically representative insect-pollinated plant species and, by so doing, to gain a better understanding of the effects of global climate change on an important plant–insect mutualistic relationship.

MATERIALS AND METHODS

Study site and natural history

This study was conducted at Hongyuan Alpine Meadow Ecosystem Research Station (Chinese Academy of Sciences), located in the eastern Qinghai Tibetan Plateau, Sichuan province (32° 48' N, 102° 33' E; 3500 m asl). The climate is cold, continental, and characterized by a short and cool spring, summer and autumn and a long cold winter. According to the data collected at the Hongyuan County Climate Station (located 5 km from the study site and at the same altitude) during 1961–2012, the annual mean temperature was 0.95 °C, with maximum and minimum monthly means of 10.2 and –5.3 °C in July and January, respectively. Data collected during the same

time period show that mean annual temperature increased by 0.29 °C per decade, with temperature increases in the non-growing season (October to April) and growing season (May to September) of 0.48 °C and 0.22 °C per decade, and increases in night-time and daytime temperature (as indicated by daily minimum and maximum temperatures, respectively) of 0.50 °C and 0.29 °C per decade, respectively. Annual mean precipitation is 744 mm, of which 80 % occurs between May and August (Wu *et al.*, 2011), and soils are typified by a high organic content (250 g kg⁻¹) and low total N (8 g kg⁻¹) and P (5 mg kg⁻¹) (Liu *et al.*, 2012).

The vegetation is dominated by grasses, such as *Kobresia uncinoides* and *Kobresia pygmaea*, and forbs, such as *Saussurea nigrescens*, *Potentilla anserina*, *Polygonum macrophyllum* and *Anemone trullifolia* var. *linearis* (Li *et al.*, 2011). Our study species, *S. nigrescens* (Asteraceae) is common throughout the study site and more generally in alpine meadows in China at altitudes ranging from 2900 to 4300 m. Plants are 15–40 cm high, bearing two to five capitula, each of which is composed of 20–55 flowers with an annular bowl-shaped nectary at the distal end of the ovary between the ovary and anthers. *Saussurea nigrescens* is a monoclinal and dichogamous species with male anthers reaching maturity earlier than stigmas, flowering from late July to August and fruiting in September before senescence. In addition to being used by native pollinators such as *Bombus filchnerae*, *Bombus humilis* and *Bombus supremus* (Macior *et al.*, 2001), *S. nigrescens* is the most important forage plant for honeybees in the region.

Warming experiment

In September 2007, 40 open-top chambers (OTCs) measuring 1 × 1 × 1 m were randomly deployed (at a minimum spacing of 3 m) in a fenced, flat area of ≈1.0 ha. The sides of 20 OTCs were covered with thin (<0.1 mm) steel screen with a mesh size of 0.2 × 0.2 mm. The other 20 OTCs were covered with clear, smooth polycarbonate sheeting, which provided a warming effect the steel screening did not (Wu *et al.*, 2011). The average transparency of the steel screen was 84 % ($n=5$) under full light conditions, slightly less than that of the polycarbonate sheet (86 %, $n=5$) in full light in mid-July ($t=0.974$, $P=0.358$). Each OTC was sunk 10–15 cm into the soil and firmly stabilized to withstand extremely windy conditions, which occur often at the site. Grazing was high in adjoining areas but large herbivores were excluded from the study site by fencing.

Measurements using a DS1921G model thermometer (Maxim Integrated Products, Sunnyvale, CA, USA) from 2007 to 2013 showed that the mean annual temperature 30 cm above ground was 0.7 (±0.05) °C higher in the warmed OTCs than in the ambient OTCs during the growing season (May to September) and was 2.7 (±0.17) °C higher in the non-growing season (October to April). Night-time (19:00 to 7:00, Beijing time) temperatures (30 cm above ground) were 2.8 (±0.38) °C higher in the warmed OTCs than in the ambient OTCs during

the period from 1 June to 31 August, when *S. nigrescens* flowered. The daytime (7:00 to 19:00) temperatures were also $1.6 (\pm 0.17)^\circ\text{C}$ higher in the ambient OTCs during the same time period (Supplementary Data Table S1). In addition, measurements made on three sunny days (between 10:00 and 14:00) showed that the air temperature difference 30 cm above the soil surface was $1.97 \pm 0.11^\circ\text{C}$, while capitulum tissue was $1.02 \pm 0.40^\circ\text{C}$ warmer in warmed compared with ambient OTCs ($n=20$ for each treatment). There was no variation in soil moisture recorded at a depth of 5 cm once every month during the non-freezing periods (April to October in 2012; Supplementary Data Fig. S1) or relative humidity between the warmed and ambient OTCs (81.6 ± 8.45 and $84.7 \pm 1.57\%$, respectively).

Nectar measurements

Floret nectar volumes and concentrations were measured for plants in the warmed and ambient OTCs from late July to early August in both 2012 and 2013, when most mature plants were observed flowering. We chose five to ten vigorous, medium-sized plants in each OTC and tagged and enclosed one capitulum (in which all florets were closed) per plant with bridal veiling to exclude insect visitors (Real and Rathcke, 1991). Previous work has shown that the nectar volume peaks when anthers become white in appearance (Mu et al., 2014) and we used this visual cue as an indicator of when to measure floret nectar volumes and concentrations (from 10:00 to 15:00 on sunny days). Nectar volume was measured for a minimum of five florets per capitulum using 1- or 5- μl micropipettes (Hirschmann Laborgeräte, Germany) and nectar (sucrose) concentration was concurrently measured with a hand refractometer (Eclipse; Bellingham and Stanley, UK) following Johnson et al. (2006). About 4500 florets were used to monitor the nectar volume and concentration.

Non-structural carbohydrate depletion

To determine whether night-time warming significantly affected leaf non-structural carbohydrate levels, we measured non-structural carbohydrate for representative leaves sampled just after sunset (when non-structural carbohydrate values were at their maximum) and leaves sampled just before sunrise (when non-structural carbohydrate values were at their minimum). The leaves were removed from plants growing in ten warmed OTCs and ten ambient OTCs on 5 and 6 August 2013. The samples were kept in a portable icebox with dry ice and taken to the laboratory, where non-structural carbohydrate levels were measured following the procedure of Hansen and Møller (1975) and Yoshida et al. (1976). We calculated the non-structural carbohydrate depletion by subtracting the non-structural carbohydrate content measured at sunset from the non-structural carbohydrate content measured at sunrise from plants growing in the same OTCs.

Plant morphology

In addition to measuring nectar volume and quantity and non-structural carbohydrate content, we tracked plant density

and flowering phenology by recording the total number of plants and the number of flowering plants in each OTC during the flowering season of both 2012 and 2013. The total number of plants per OTC was taken as plant density. The flowering onset and offset times were both defined as the dates when 10 of all plants were flowering (Sun and Frelich, 2011). The percentage of flowering plants was calculated with respect to the total number of flowering plants divided by the total number of plants in each OTC. We investigated 20 ambient OTCs and 20 warmed OTCs in both years using these protocols.

We also measured plant height and aboveground biomass for a minimum of three plants per OTC. Plant height was measured from the soil surface to the apex of the terminal shoot to the nearest 0.5 cm (Nagashima et al., 1995). We then harvested aboveground parts, which were dried to constant mass and weighed in the laboratory. The biomass of leaves, stems and capitula (fruits) was measured per plant, as was the number of capitula. Ripened capitula were removed and the number of aborted ovules and sound seeds was recorded. The seeds were weighed (to 0.1 mg) for each capitulum. Total seed number per plant was calculated as the product of seed number per capitulum and the number of capitula per plant. Seed set was calculated as the percentage of ovules that matured into sound seeds. We used 420 plants to measure these morphological and reproductive features.

Statistical analyses

Data for each of the measured traits were tested for normality before analysis. The floret nectar volume was \log_{10} -transformed and the proportion of flowering plants per OTC was arcsine-transformed to achieve normality. The differences in nectar volume and concentration, floret number per capitulum, the number of capitula per plant, plant density, plant height, and aboveground biomass between treatments were determined using three-way mixed ANOVA with OTC identity as the random factor and warming and study year as the fixed factors. The treatment differences in plant density, proportion of flowering plants, nectar productivity and flowering phenology were determined using two-way ANOVA with study year and warming as the fixed factors. Once a significant warming effect was detected, *post hoc* Tukey's tests were used to determine the difference in each study year. The difference in non-structural carbohydrate depletion between warmed and ambient OTCs were assessed with *t*-tests. In addition, correlation analyses were conducted to determine the relationships among the above parameters across treatments for both study years.

Plant nectar productivity was gauged on the basis of nectar volume per floret (FNV), floret number per capitulum (FN), capitulum number per plant (CN), plant density (total number of plants [TNP] per OTC) and the proportion of flowering plants (PFP): plant nectar productivity = $\text{FNV} \times \text{FN} \times \text{CN} \times \text{TNP} \times \text{PFP}$. The difference in plant nectar productivity between warmed and ambient OTCs was determined using two-way ANOVA with warming and study year as the fixed factors. In order to determine the extent to which the five different variables contributed to the variation in plant nectar productivity, variance component analysis (varcomp in R; www.r-project.org) was used to partition plant nectar productivity variance

among the variables. Bootstrapping was used to calculate the 95 % confidence intervals of variance components for each factor. We constructed a data set by randomly sampling the data points from the original data set with replacement, and then the variance component for each factor was calculated by the method mentioned above. This procedure was repeated 1000 times and each result was stored. The 95 % confidence interval was calculated as the values corresponding to 0.025 and 0.975 percentiles. All statistical analyses were performed in R (www.R-project.org).

RESULTS

Artificial warming significantly reduced floret nectar volume, floret number per capitulum, capitulum number per plant, proportion of flowering plants and plant density in both study years (Table 1; Fig. 1).

The proportion of flowering plants was significantly smaller in 2012 than 2013 (Supplementary Data Table S2), but no significant difference was found in floret nectar volume, capitulum number per plant or plant density (Tables 1 and 2).

Although the difference in the nectar concentration was indistinguishable between warmed and ambient OTCs in both study years (Table 1; Fig. 1), nectar productivity was significantly lower (by >90 %) in the warmed OTCs compared with the ambient OTCs in both years (Tables 1 and 2). Moreover, nectar productivity was significantly smaller in 2012 than 2013 (Table 2; Supplementary Data Table S2).

Warming had no impact on flowering phenology; the flowering onset time and flowering offset time were indistinguishable between warm and ambient OTCs in both study years (Tables 1 and 2). Likewise, warming had no significant effect on seed set, plant height or aboveground biomass in both years (Tables 1 and 2). Nevertheless, warming significantly reduced total seed number per plant and seed mass per plant in both years (Tables 1 and 2).

Depletion of leaf non-structural carbohydrates was significantly higher in the warmed than ambient OTCs (Fig. 2). This was likely linked with the reduction of floret nectar volume, as indicated by the significantly negative relationship between depletion and nectar volume (Fig. 3). Floret nectar volume was positively correlated with capitulum number per plant, which was positively associated with aboveground plant biomass (Supplementary Data Table S3). Moreover, plant nectar production was positively correlated with floret nectar volume, proportion of flowering plants and capitulum number per plant (Supplementary Data Table S3).

Variance component analysis showed that the five factors (floret nectar volume, floret number per capitulum, capitulum number per plant, total number of plants and proportion of flowering plants) could explain >90 % of the variation in plant nectar productivity (Table 3). Floret nectar volume accounted for most of the variation (60 %), followed by the proportion of flowering plants (11 %) and capitulum number per plant (10 %).

DISCUSSION

We show that warming (most markedly elevated winter and summer night-time temperatures) had negative impacts on

TABLE 1. Summary of ANOVA for the effects of warming (ambient versus warmed) and year (2012 and 2013) on nectar production and concentration, vegetative and reproductive traits, flowering phenology of the Tibetan alpine Asteraceae (*S. nigrescens*). The open top chamber (OTC) was assigned as a random factor in the analyses, except for flowering phenology, plant density, proportion of flowering plants and nectar productivity

	d.f.	F-value	P-value
Floret nectar volume			
Warming	1	74.080	<0.001
Year	1	0.698	0.406
Warming × year	1	0.253	0.617
Residuals	75		
Nectar concentration			
Warming	1	0.560	0.457
Year	1	0.181	0.672
Warming × year	1	0.623	0.432
Residuals	75		
Floret number per capitulum			
Warming	1	17.419	<0.001
Year	1	3.851	0.053
Warming × year	1	0.404	0.527
Residuals	75		
Capitulum number per plant			
Warming	1	97.588	<0.001
Year	1	0.678	0.413
Warming × year	1	0.237	0.628
Residuals	75		
Nectar productivity			
Warming	1	200.941	<0.001
Year	1	6.206	<0.05
Warming × year	1	3.043	0.085
Residuals	76		
Plant height			
Warming	1	0.080	0.775
Year	1	0.027	0.870
Warming × year	1	0.095	0.759
Residuals	75		
Aboveground vegetative biomass			
Warming	1	0.156	0.694
Year	1	0.754	0.388
Warming × year	1	0.015	0.904
Residuals	75		
Seed number per plant			
Warming	1	48.30	<0.001
Year	1	0.010	0.922
Warming × year	1	0.910	0.343
Residuals	75		
Seed set			
Warming	1	2.299	0.134
Year	1	0.739	0.393
Warming × year	1	0.102	0.751
Residuals	75		
Seed mass per plant			
Warming	1	134.377	<0.001
Year	1	5.045	<0.05
Warming × year	1	2.359	0.129
Residuals	75		
Flowering onset time			
Warming	1	1.176	0.282
Year	1	2.305	0.133
Warming × year	1	0.228	0.635
Residuals	76		
Flowering offset time			
Warming	1	3.358	0.071
Year	1	0.089	0.766
Warming × year	1	0.089	0.766
Residuals	76		
Plant density			
Warming	1	16.84	<0.001
Year	1	0.144	0.705
Warming × year	1	0.827	0.366
Residuals	76		
Proportion of flowering plants			
Warming	1	25.413	<0.001
Year	1	5.856	<0.05
Warming × year	1	0.406	0.526
Residuals	76		

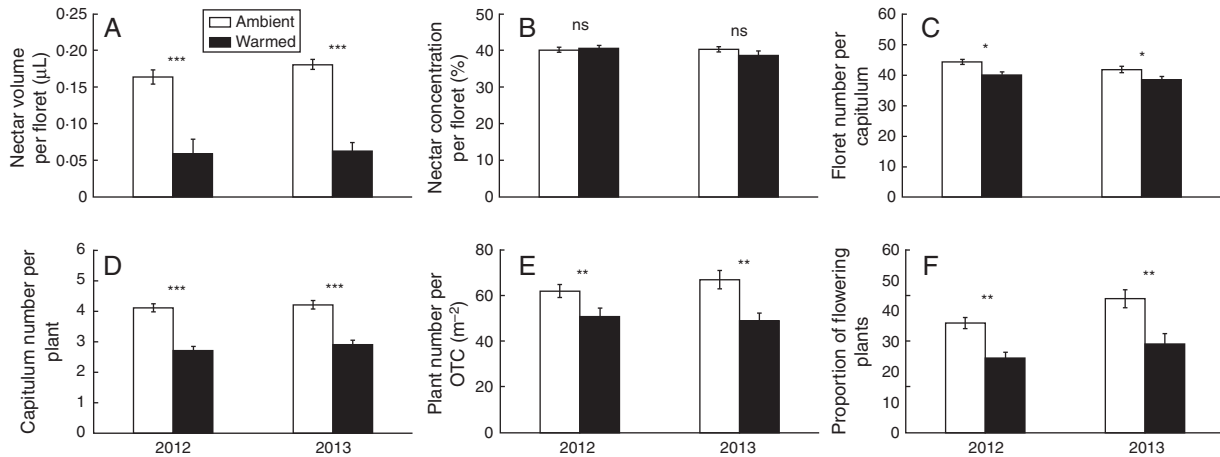


FIG. 1. Floret nectar volume and concentration (A, B), floret number per capitulum (C), capitulum number per plant (D), plant number per open-top chamber (OTC) (E) and proportion of flowering plants (F) for warmed and ambient OTCs in 2012 and 2013. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ (one-way ANOVA).

TABLE 2. Means (\pm s.e.) of vegetative and reproductive traits, flowering phenology of the Tibetan Asteraceae *Saussurea nigrescens* between ambient and warmed open top-chambers (OTCs) in 2012 and 2013. All means are for the individual plant level. Plant height, aboveground vegetative biomass and capitulum number per plant were all based on single measurements per plant

	Ambient OTCs	Warmed OTCs
2012		
Plant height (cm)	29.03 \pm 0.56 ^a	30.14 \pm 0.68 ^a
Aboveground vegetative biomass (g)	1.49 \pm 0.05 ^a	1.38 \pm 0.06 ^a
Seed number per plant	78.10 \pm 4.63 ^a	49.73 \pm 5.10 ^b
Seed set (%)	61.80 \pm 1.62 ^a	66.60 \pm 2.64 ^a
Seed mass per plant (mg)	185.00 \pm 9.33 ^a	77.00 \pm 8.40 ^b
Flowering onset time	212.10 \pm 0.69 ^a	211.20 \pm 0.55 ^a
Flowering offset time	228.50 \pm 0.56 ^a	227.20 \pm 0.76 ^a
Nectar productivity (μ l)	668.32 \pm 63.64 ^a	59.74 \pm 13.54 ^b
2013		
Plant height (cm)	28.86 \pm 0.63 ^a	30.74 \pm 0.70 ^a
Above-ground vegetative biomass (g)	1.52 \pm 0.06 ^a	1.46 \pm 0.10 ^a
Seed number per plant	83.07 \pm 5.08 ^a	45.67 \pm 3.94 ^b
Seed set (%)	65.00 \pm 2.42 ^a	68.00 \pm 3.33 ^a
Seed mass per plant (mg)	225.80 \pm 14.79 ^a	84.40 \pm 9.20 ^b
Flowering onset time	211.00 \pm 0.46 ^a	210.60 \pm 0.58 ^a
Flowering offset time	228.10 \pm 0.49 ^a	227.20 \pm 0.48 ^a
Nectar productivity (μ l)	875.67 \pm 72.91 ^a	96.29 \pm 25.64 ^b

Different letters after means denote significant differences in values ($P < 0.05$; two-way ANOVA followed by *post hoc* Tukey test.

All data are normally distributed (Shapiro-Wilk test, $P > 0.1$ for each OTC); $n = 20$ for both ambient and warmed treatments.

nectar yield and thus on the likely consequences for plant–pollinator interactions during the peak summer flowering period of *S. nigrescens*. An important ancillary observation is that the reduction in nectar was associated with the depletion of non-structural carbohydrates, as indicated by the negative relationship between the depletion of non-structural carbohydrates and floret nectar volume across OTCs. Night-time warming can increase nocturnal respiration and thus deplete carbohydrates (Ryan, 1991; Griffin et al., 2002; Wan et al., 2009) that would otherwise be allocated to the production of nectar. Our data

also show that asymmetric warming has other negative effects, such as reductions in flower number per capitulum, capitula number per plant, the proportion of flowering plants and plant density. These phenomena, which are likely linked to devernalization and competition among viable plants, have negative effects on the resources plants make available to pollinators and thus possibly on the density and foraging behaviour of pollinators (Hanley et al., 2008, 2014; Gilman et al., 2012).

Large differences in daytime and night-time temperatures are known to favour nectar production in many other species (McDade and Weeks, 2004; Wolff, 2006). For example, Jakobsen and Kristjánsson (1994) found that low night-time temperatures (~ 10 °C) can increase nectar production but only with increased daytime temperatures. Conversely, when temperatures exceed a threshold daily maximum, nectar production can decrease, often drastically (Nicolson, 1995; Petanidou and Smets, 1996). The underlying mechanism for this phenomenon is that high daytime temperatures can enhance photosynthesis and carbon gain, whereas low night-time temperatures tend to reduce respiration and the depletion of stored carbohydrates. As a result, non-structural carbohydrate content and flower or fruit quality (as gauged by sugar content; Percival, 1961) are often improved by increasing the night–day temperature difference. In our case, asymmetric warming greatly increased the depletion of non-structural carbohydrate content and decreased nectar volume per floret despite the lack of significant difference in illumination, relative humidity or soil moisture between warmed and ambient open top chambers.

Asymmetric warming also affects vernalization and thus population demographics. Cold winters are often necessary for flower formation in perennial herbaceous species growing in temperate regions, because many of these species flower only after they experience an extended period of cold temperatures (Bernier et al., 1981; Michaels and Amasino, 2000), likely as an adaptation to a growing season and the benefits conferred by rapid flowering and seed production (Aarssen and Jordan, 2001). For these species, even a slight increase in winter temperatures can have significant negative consequences (Chouard, 1960; Scaven and Rafferty, 2013). For example, Yu et al.

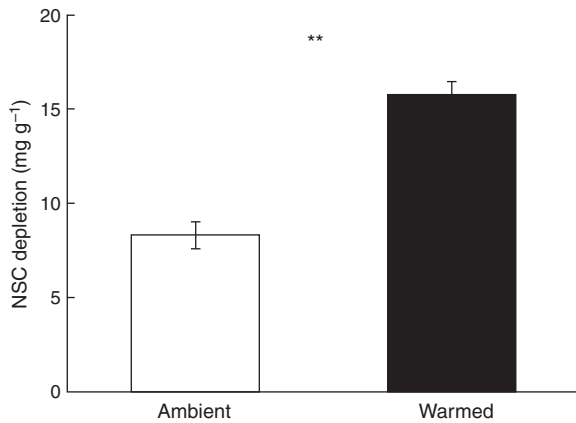


Fig. 2. Non-structural carbohydrate (NSC) depletion between ambient and warmed open-top chambers in 2013. $**P < 0.01$ (one-way ANOVA).

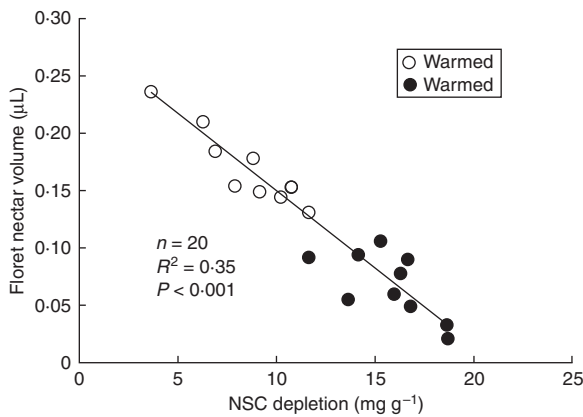


Fig. 3. Regression of floret nectar volume on non-structural carbohydrate (NSC) depletion across open-top chambers.

TABLE 3. Variance components of plant nectar productivity (PNP) for five factors

Factor	Variance of the response (%) (95% CI ^a)
Floret nectar volume	60 (36, 83)
Floret number per capitulum	0.91 (2.21e-03, 7.58)
Capitulum number per plant	10 (0.54, 26)
Total number of plants per OTC	7.4 (1.6, 15)
PFP proportion of flowering plants	11 (3.3, 19)
Residuals	9.8 (5.6, 13)

^aCalculated by bootstrapping.

(2010) report that the ‘spring greening’ phenology has been delayed in the latest two decades due to winter temperature increases, while Liu *et al.* (2012) also report reductions in the proportion of flowering shoots and seed output for a variety of herbaceous species subjected to artificial warming. This observation is consistent with the decline in the proportion of flowering plants and the number of capitula per plant observed for our study species and are likely a result of weakened vernalization

due to the winter warming (Scaven and Rafferty, 2013). This explanation is also consistent with many previous reports in that higher than normal temperatures result in what has been called devernization (Bernier *et al.*, 1981; Saavedra *et al.*, 2003; Bokhorst *et al.*, 2008), which depresses flower formation (Chouard, 1960; Hideyuki and Takashi, 2003; Liu *et al.*, 2012).

In this context, other potential factors appear not to have contributed to the phenomenon reported here. For example, studies frequently indicate that some species flower only after they gain a threshold height or biomass and only when the timing of flower bud formation is appropriate. Nevertheless, we did not find a significant shift in plant phenology or plant growth between warmed and ambient chambers, possibly because the warming effect on plant phenology might not have emerged during the study years. Furthermore, early physiological experiments show that water stress can induce flowering, presumably as a result of an evolutionary advantage of producing seeds before ageing (Southwick and Davenport, 1986; Ekanayake *et al.*, 1988). Our data show that this potential stress had no effect (as indicated by comparisons of plant height and biomass). In addition, the effect of the physical setting on pollinators should be similar between warmed and ambient chambers, as indicated by the similar levels of seed set between treatments. This can be attributed to the facts that the OTCs (for both warmed and ambient treatments) served as similar physical barriers preventing migration of most insect species (due to small mesh size of the ambient OTCs) and that the major pollinator species for *S. nigrescens*, the domesticated honeybee (*Apis mellifera*), was extremely abundant in the study site (Mu *et al.*, 2014).

Artificial asymmetric warming also resulted in a significant decline in plant density, except for the reduction in floret nectar volume and flowering potential. Decreased seed production per plant might have contributed to the reduction in plant density in *Saussurea*, because seedling density was often positively associated with seed density in the study region (Xi *et al.*, 2015). The reduction in seed production is likely due to the negative effect of warming on capitulum/floret number per plant, but it cannot be accounted for by the indistinguishable difference in bee visitation rates and seed set.

In summary, our results show that asymmetric warming can reduce nectar provision in *S. nigrescens* via reduced nectar production of individual florets, fewer florets per capitulum and capitula per plant, and a lower proportion of flowering plants within a reduced overall plant density. We speculate that climate change will likely have increasingly negative effects on plant-pollinator interactions (Roulston and Goodell, 2011; Scaven and Rafferty, 2013), but highlight the pressing need for future research to address how climate-induced changes in plant physiology and resource allocation affect pollinator reward provision more generally.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: monthly means of soil moisture for the duration of the experiment. Table S1: differences in the temperature 30 cm above ground in warmed and ambient open-top chambers from 2007 to 2013. Table S2: results of Tukey *post hoc* tests showing the effects of

warming and year on nectar production and concentration, vegetative and reproductive traits and flowering phenology. **Table S3**: matrix of the correlation coefficients among the variables studied.

ACKNOWLEDGEMENTS

We thank Jiyan Zhao, Yan Li, Jie Xiong, Hongli Chen, Yongpin Li, Rui Cao, Yangheshan Yang, Xincheng Li and Kai He for field and laboratory assistance. This study was funded by the 973 Program (2013CB956302) and the National Science Foundation of China (31270513, 31100397 and 31325004).

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