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Experimental test for adaptive differentiation of ginseng populations reveals complex response to temperature

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• *Background and Aims* Local climatic adaptation can influence species' response to climate change. If populations within a species are adapted to local climate, directional change away from mean climatic conditions may negatively affect fitness of populations throughout the species' range.
• *Methods* Adaptive differentiation to temperature was tested for in American ginseng (*Panax quinquefolius*) by reciprocally transplanting individuals from two populations, originating at different elevations, among temperature treatments in a controlled growth chamber environment. Fitness-related traits were measured in order to test for a population × temperature treatment interaction, and key physiological and phenological traits were measured to explain population differences in response to temperature.
• *Key Results* Response to temperature treatments differed between populations, suggesting genetic differentiation of populations. However, the pattern of response of fitness-related variables generally did not suggest 'home temperature' advantage, as would be expected if populations were locally adapted to temperature alone.
• *Conclusions* Failure consistently to detect a 'home temperature' advantage response suggests that adaptation to temperature is complex, and environmental and biotic factors that naturally covary with temperature in the field may be critical to understanding the nature of adaptation to temperature.

Key words: Adaptive differentiation, American ginseng, climate change, demography, growth chamber, local adaptation, *Panax quinquefolius*, temperature.

INTRODUCTION

Local adaptation refers to intraspecific genetic differentiation of populations such that local genotypes have higher fitness in their home sites relative to foreign genotypes (Futuyma and Moreno, 1988; Linhart and Grant, 1996; Kawecki and Ebert, 2004; Savolainen *et al.*, 2007). Heterogeneous selective forces within a species' range promote specialization of populations to local environmental conditions (Futuyma and Moreno, 1988; Kawecki and Ebert, 2004). Evolution of locally adapted populations occurs when gene flow and genetic drift are too weak to counteract selection (Futuyma and Moreno, 1988; Kawecki and Ebert, 2004). If selection is sufficiently strong, adaptive differentiation can occur even when gene flow is high (Futuyma and Moreno, 1988; Linhart and Grant, 1996; Kawecki and Ebert, 2004; Antonovics, 2006). For local adaptation to occur, there is a requisite fitness trade-off, such that morphological or physiological traits that increase fitness in one environment incur a fitness cost in a different environment. Adaptive differentiation of populations would not occur if a single genotype could gain superiority in all environments (Futuyma and Moreno, 1988; Kawecki and Ebert, 2004). Phenotypic plasticity has traditionally been viewed as an alternative to local adaptation, allowing a particular genotype to maintain high fitness by phenotypic adjustment to a wide range of environmental conditions. Recent research suggests that phenotypic plasticity, itself a

trait, may vary among populations within a species' range, and may be a proximate step in local adaptation by allowing a species to produce distinct phenotypes in differing environments on which selection can act (Matesanz *et al.*, 2010; Riis *et al.*, 2010; Ward *et al.*, 2012).

Reciprocal transplant experiments are the standard test for local adaptation (Futuyma and Moreno, 1988; Linhart and Grant, 1996; Kawecki and Ebert, 2004). In such experiments, populations are reciprocally transplanted among sites to compare performance of 'local' vs. 'non-local' genotypes in each population's home site. Reciprocal transplant experiments partition environmental and genetic components of population performance across an environmental gradient. For instance, if populations within a species exhibit a solely plastic response to the environment, there will be no apparent genetic ('population') effect, and vice versa in the case of populations for which performance is strictly genetically controlled. A population × environment interaction, such that populations demonstrate a 'home site' advantage, indicates local adaptation (Kawecki and Ebert, 2004). Reciprocal transplant experiments can be modified to explore adaptive differentiation of populations to specific environmental factors. In such an experiment, gardens comprised of various populations or genotypes are established across an environmental gradient (Linhart and Grant, 1996). A differential, typically clinal, response among populations in terms of morphological, physiological, reproductive or phenological traits along the

environmental gradient indicates intraspecific adaptive differentiation with respect to the environmental factor of interest (Linhart and Grant, 1996; Savolainen *et al.*, 2007). Such experiments have demonstrated population-level adaptation to a variety of selective forces, including, but not limited to, heavy metals and other edaphic factors, light regime and climate (Linhart and Grant, 1996).

Specialization to local conditions may be adaptive in a stable environment, but can become a fitness liability if environmental conditions change (Holt and Gaines, 1992; Holt and Barfield, 2008; Holt, 2009). For species adapted to local climate, directional climate change may push populations from fitness peaks, and threaten long-term persistence (Davis and Shaw, 2001; Davis *et al.*, 2005). Past transplant studies along elevational or latitudinal gradients, which served as a proxy for climate gradients, have shown that alteration in climate regime can levy large fitness costs (Etterson, 2004; Savolainen *et al.*, 2007). For instance, Etterson (2004) demonstrated that non-local genotypes of *Chamaecrista fasciculata* experienced up to a 96 % reduction in seed production compared with local genotypes in a transplant experiment across a latitudinal gradient. If future climatic conditions exceed the population-level climatic niche, climate change will initiate decline of extant populations throughout a species' range in the absence of gene flow unless populations acclimate or adapt to the novel climatic conditions (Souther and McGraw, 2011). Because the population-level climatic niche breadth is reduced relative to the range of climatic conditions occupied by the species, population decline will result following a smaller change in climate than would be expected given the spatial distribution of the species. While *in situ* adaptation to future climate is possible, selection may lower genetic variation within populations of locally adapted species, making rapid adaptation less probable. In this way, climate change may threaten even widespread species when populations have specialized to local climatic conditions.

In a previous field study, we used census data collected over a 6–12 year period for 12 populations of American ginseng that occurred across a broad geographic range to examine patterns of demographic response to interannual temperature variation (Souther and McGraw, 2011). There was a consistent, population-level, parabolic relationship between population growth rate (λ) and temperature, such that as annual temperatures deviated from site-specific means, λ-values decreased, suggesting local adaptation to temperature (Souther and McGraw, 2011). Even when multiple warm or cool years occurred in sequence, population growth rates did not increase, suggesting acclimation to temperature was not occurring, and therefore the reduction in λ was based on adaptation to the local mean climate. In the current study, we conduct a reciprocal transplant-type experiment in a growth chamber environment using two populations of ginseng, one from a high elevation site and one from a mid-elevation site. Using growth chambers to manipulate mean temperature, while maintaining other environmental factors constant, we could isolate temperature effects, in order to ask the following questions. (1) Is there a genetic basis to differential population response to temperature? (2) Do the direct effects of temperature explain the pattern of local adaptation observed in Souther and McGraw (2011)? We expected that response to temperature

would be genetically based, and that temperature would directly affect fitness-related traits, such that performance would be maximized at temperature conditions approximating each population's home site.

MATERIALS AND METHODS

Study species

American ginseng (*Panax quinquefolius* L.) is a perennial herb endemic to the understorey of the eastern deciduous forest of North America (Anderson *et al.*, 1993; Anderson, 1996; Robbins, 2000; McGraw *et al.*, 2003). The harvest of ginseng annually generates millions of dollars in supplemental income for Appalachian families (Bailey, 1999; Robbins, 2000). Concerns that ginseng was becoming increasingly rare due to overharvest prompted the 1975 listing of ginseng on Appendix II of the CITES (Convention on International Trade in Endangered Species of Fauna and Flora) treaty (Robbins, 2000). Harvest of ginseng is currently regulated by the US Fish and Wildlife Service (Robbins, 2000).

Ginseng plants typically develop between one and four palmately compound leaves, which arise in a whorl from a single, aerial sympodium. The plant's below-ground parts consist of a taproot, the primary storage organ, and a rhizome. Ginseng seedlings possess a single leaf, and several years may pass before acquiring additional leaves. Only as 'juveniles' (two-leaved plants) is reproduction possible, though seed production at this stage is infrequent. Once plants produce three leaves, reproduction increases as a function of leaf area (McGraw and Furedi, 2005; Souther and McGraw, 2011). Charron and Gagnon (1991) first modelled ginseng population dynamics, using leaf number to delineate stages in four populations of ginseng in southern Quebec. Subsequent research built on this demographic model by using leaf area in conjunction with leaf number to define stages, and incorporating seed bank dynamics in models of population growth (McGraw and Furedi, 2005; Souther and McGraw, 2011). Ginseng has a mixed mating system, and does not reproduce vegetatively or by apomixis (Lewis and Zenger, 1983; Schlessman, 1985). Ginseng berries are red and fleshy, and contain between one and three seeds. Seeds are believed to be primarily gravity dispersed, though caching by chipmunks has been documented, and other animals, such as birds, are potential dispersers (Van der Voort, 2005). Studies of genetic variation in natural populations find low within-population genetic variation, and high among-population genetic variation (Cruse-Sanders and Hamrick, 2004; Grubbs and Case, 2004).

Source populations

To test for a genetically based differential response to temperature, we selected populations of ginseng from sites differing in mean temperature conditions. Using elevation as a proxy for temperature, two populations, occurring at a high and a mid-elevation site (780 and 340 m, respectively) and separated by approx. 70 km were located, marked and mapped in northern West Virginia in autumn 2008. Both sites were north facing and characterized by mixed mesophytic tree species. However, the shrub layer was notably different

between the two sites; at the high elevation site, the shrub layer was sparse and consisted mostly of spicebush (*Lindera benzoin*), whereas the shrub layer at the low elevation site was dense and comprised of pawpaw (*Asimina triloba*). Populations also differed in terms of total growing season (15 April–30 September) precipitation. The high elevation site received a mean of 69·6 cm of rainfall, and the mid-elevation site a mean of 55·8 cm. Precise locations are withheld to prevent targeted harvest of remaining individuals at each site, as well as nearby populations. Herein, we refer to the high elevation population as the LowT Population, and the mid-elevation population as the MedT Population.

Transplant procedure

In April 2009, prior to spring emergence, 156 roots with attached rhizomes ($n = 96$, LowT Population; $n = 60$, MedT Population, respectively) were carefully excavated, cleaned of soil, weighed and aged (Anderson, 1993). In an experimental study of gene flow, pollen travelled up to 100 m from adult ginseng plants (Hackney, 1999). Based on this number, we collected all individuals separated by <100 m, and surveyed a 100 m zone around the perimeter of the population. The total area over which plants were collected was approx. 200 m × 200 m. To prevent transport of soil-borne pathogens to growth chamber facilities, roots were sterilized using a 10 % bleach solution (Hackney, 1999). Cleaned roots were individually wrapped in moist paper towels, placed inside plastic bags, and transported on ice in coolers to growth chamber facilities at the McGill Phytotron in Montreal, Quebec, Canada. Roots were transplanted to 19 cm diameter × 18 cm deep Fiber Grow nursery pots (MYE Canadian Operations Incorporated) filled with growth medium (see below) within 48 h of packaging the roots for transport.

Growth medium

We collected soil from each population's site and homogenized equal parts of these soils. We combined soil from both sites in order to maintain uniform soil composition among treatments that did not provide either population with an *a priori* 'home site' advantage with respect to edaphic factors, while replicating soil conditions found in the field. To reduce risk of introduction of soil-borne pathogens into the growth chamber facilities, soil was sterilized by autoclaving. Ginseng typically occurs on well-drained soils, particularly on sloped terrain (Anderson *et al.*, 1993). To replicate this in a growth chamber environment, sterile perlite and sand in equal parts were added to the soil mixture, such that the perlite–sand mixture comprised 50 % of the growth medium.

Temperature treatments

To set growth chamber conditions, daily 30-year (1971–2000) mean maximum and minimum temperature data were obtained from two weather stations located <25 km from each population's site (Supplementary Data Table S1). To account for elevation-related temperature differences between climate stations and source population sites, temperature data were corrected using the saturated adiabatic lapse rate

($-5 \,^{\circ}\text{C km}^{-1}$). Three temperature treatments were created: the low treatment corresponded to mean temperature conditions at the LowT Population (mean growing season temperature = 16·4 °C), the medium treatment corresponded roughly to the MedT Population site (LowT Population temperature +3 °C; mean growing season temperature = 19·4 °C), and the high temperature treatment represented 'future' climatic conditions projected by climate models (LowT Population temperature + 6 °C; mean growing season temperature = 22·4 °C). Climate models project a mean global temperature increase between 1·1 and 5·8 °C by the end of the century, thus the high temperature treatment (+ 3 °C for the MedT Population; + 6 °C for the LowT Population) bracketed several warming scenarios that ranged from mid- to high severity in terms of extent of temperature increase (IPCC, 2007).

Growth chamber parameters

Temperatures in the growth chambers were adjusted on a weekly basis to parallel seasonal temperature change experienced by source populations in their natural habitat. Daily maximum and minimum temperatures corresponded to weekly mean maximum and minimum temperatures for each temperature treatment (low, medium and high). Over each 24 h period, growth chamber temperatures were gradually ramped between temperature minima and maxima over a 6 h period, and remained at both the daily maximum and minimum temperature for 6 h. Daylength also changed on a weekly basis, and corresponded to the average weekly daylength in West Virginia. Light levels were set at 40 μmol m^{-2} s^{-1}, simulating understorey light conditions (Kota, 2005). Understorey plants (>0·05 m) experience naturally high carbon dioxide levels that vary substantially during the course of the day and throughout the growing season (Bazzaz and Williams, 1991). Carbon dioxide levels for all growth chambers were set at 410 ppm, a level that is approx. 20 ppm above ambient atmospheric conditions, and that corresponds to CO_2 levels near the forest floor in summer (Bazzaz and Williams, 1991). Seventy per cent humidity was maintained within the growth chambers. We did not want to confound the effects of temperature with soil moisture, so plants were watered every 1–3 d as necessary to maintain moist soil conditions. Consequently, plants in the warmer temperature treatments were watered more frequently.

Experimental design

Plants from each population were randomly assigned to each of the three temperature treatments ($n = 31$, LowT Population; $n = 20$, MedT Population), and placed into the corresponding growth chamber. To control for growth chamber differences, treatments and corresponding plants were rotated among growth chambers on a monthly basis. To account for environmental gradients within growth chambers, plants were rotated within growth chambers weekly.

Plants received treatments for two consecutive growing seasons (approx. 20 April–30 September). After the first growing season, plants were overwintered in a lath house at the Gault Nature Reserve (www.mcgill.ca/gault/). We buried plants, within their pots, in sand, flush to pot soil level.

To insulate against the risk of extreme winter temperatures, pots were covered with fibreglass insulation and heavy plastic sheeting after complete foliar dehiscence. At the onset of year 2 of the experiment (April 2010), we fertilized all plants with 200 mL of 50 % Hoagland's 20-20-20 nutrient solution to simulate spring influx of nutrients from litter decomposition. This was the only nutrient application in the course of the two-season experiment.

Survival

At the end of the experiment, the fate of death was assigned when a root was completely missing. If a root was present, but there was no shoot, the plant was considered dormant, but not dead.

Growth measurements

During the first week of July in both 2009 and 2010, after ginseng had terminated leaf expansion, we measured leaf area. Leaf area was estimated from foliar measurements, using a previously derived regression equation that related leaflet length and width to leaf area (Souther and McGraw, 2010). At the termination of the experiment, roots were separated from shoots, cleaned, and fresh mass was obtained. Fresh mass of above-ground biomass (leaves, sympodium and peduncle) was measured. Subsequently, above-ground biomass was dried at 65 °C for 48 h and weighed.

We calculated the relative growth rate of leaf area (RGR_{LA}) as:

$$RGR_{LA} = [\ln(LA_2) - \ln(LA_1)]/(t_2 - t_1)$$

where LA is leaf area and t is time (McGraw and Garbutt, 1990). Relative growth rate of the root (RGR_{ROOT}) was calculated using this same formula, with fresh root mass in place of LA.

Reproductive measurements

In both 2009 and 2010, we also counted floral buds during the first week of July as well as ginseng berries and seeds at the end of September. The number of seeds per bud was calculated by dividing the total seed number per plant by the total bud number per plant. In 2010, we measured berry mass per seed-producing plant. We calculated mean berry mass per seed by dividing the bulk berry mass by seed number for each plant.

Physiological measurements

Photosynthesis and respiration were measured using a Li-Cor 6400 Portable Photosynthesis System over a 5 d period in July 2010. During this time, growth chambers were programmed so that temperatures remained constant at the weekly temperature maximum and minimum, except during a 1 h period in which temperatures changed between these two states; the maximum temperature occurring during the day [0600–1800 h EDT (Eastern Daylight Time)] and the minimum temperature occurring during the night (1900–0500 h EDT). We measured photosynthesis and respiration for ten randomly selected individuals from both populations in each temperature treatment ($n = 10$).

During this week, 'sunrise' in the chambers occurred at 0500 h, and 'sunset' at 1900 h. Photosynthesis was measured between 0700 and 1200 h and from 1300 to 1800 h EDT. To avoid systematic error in photosynthetic measures caused by diurnal variation in photosynthetic rate, the order of photosynthetic measurements within and among temperature treatments was randomized. Net photosynthesis (A_{max}) was measured at both ambient light levels (40 μmol m^{-2} s^{-1}) and light saturation (500 μmol m^{-2} s^{-1}); the saturating light level was determined using photosynthetic light response curves for ginseng (Jochum *et al.*, 2007). Carbon dioxide levels were kept constant at 380 ppm. Respiration was measured using the same procedures as above, but in the absence of light, between 2100 and 0000 h.

Phenological measurements and allocation

Emergence was scored on 8 May 2009 and 20 April 2010 by assessing plants against six phenophases (one being the least developed and six being the most developed) that differed in degree of sympodium development, erectness and foliar expansion. In July 2009 and 2010 while performing bud counts, we measured flowering phenology by tallying the number of floral buds in each of three flowering states (pre-anthesis, anthesis and post-anthesis) per plant. During seed counts in September in 2010, we scored senescence level. Plants that had little to no browning (brown leaf area <5 % total leaf area) were assigned a senescence level of 1, those with mild foliar browning (5–25 % of total leaf area) were assigned a level of 2, those with severe browning (26–75 % of total leaf area) a level of 3, and those that were nearly totally brown (75–100 % of total leaf area) a level of 4.

At the time of harvest in September 2010, the root to shoot ratio was calculated by dividing the fresh mass of the root and rhizome by the fresh mass of the shoot (leaves, sympodium and peduncle). Although dry masses would have been preferable, the valuable roots were conserved for future research.

Analyses

For all analyses, model effects included temperature, population and temperature × population. As is the case with many plant species, size strongly influences survival and reproduction in ginseng, and there were initial differences in plant size among individuals and between the two study populations. For this reason, a metric of size was used as a covariate in most analyses (Supplementary Data Table S2). For presence/absence, survival and growth-related analyses, we used the covariate initial root mass. Because reproduction varies as a function of leaf area (Mooney and McGraw, 2009), leaf area corresponding to the year of reproduction was used as a covariate in all analyses of reproductive traits (e.g. for the response variable bud production year 1, the covariate was leaf area year 1). Plants grew to large sizes in the second year of the study, and taller plants may have competitively suppressed smaller individuals via shading. To account for this, sympodium height was also included as a covariate in year 2 analyses

of reproduction variables. If the covariate was not significant, the analysis was rerun with the covariate removed.

Individuals were excluded from analyses if they (1) incurred damage during the transplantation process or subsequently which resulted in foliar deformities in year 1 of the experiment; or (2) senesced early, leading to desiccation of foliar material which rendered comparisons involving fresh weight of above-ground biomass invalid (Supplementary Data Table S2).

For all analyses with a continuous dependent variable, a two-way analysis of variance (ANOVA) was used to analyse data. A *G*-test was used for analyses in which the dependent variable was nominal and, where necessary, data were transformed to improve normality (Supplementary Data Table S2).

RESULTS

Presence/absence and survival

Overall mortality was low for the experiment; only four plants (3·2 % of all individuals) died during the transplant process and over the course of the study, so there were no significant effects of population, temperature or their interaction on survival (Table 1).

Growth

There was a tendency for the effect of temperature on RGR_{LA} to differ between populations, but no differential treatment effect was found for RGR_{ROOT} (Table 1). Populations did differ in RGR_{ROOT}, the MedT Population accumulating greater biomass compared with the LowT Population (Table 2).

Reproduction

There was a tendency for the effect of temperature on flower bud number to differ between populations in 2009, a pattern that was statistically significant in 2010 (Table 1). While the two populations responded differently to temperature in terms of bud number, they did so in an unexpected manner: the LowT population showing greater bud number at high temperature, while the MedT population was unresponsive (Fig. 1). Temperature influenced bud production in 2010, with plants in the medium temperature treatment producing 16 % more buds per plant compared with plants in the high temperature treatment, which produced the least number of buds (Supplementary Data Table S3). In 2009 only, populations differed in the number of buds produced, such that plants from the MedT Population produced a greater number of buds per plant than the LowT Population (Table 2).

While we expected that the effect of temperature on metrics of seed production would differ between populations, this was not the case (Table 1). In general, temperature alone influenced measures of seed production, and in all cases seed production was lowest in the high temperature treatment (Table 2). In both 2009 and 2010, temperature affected the number of seeds per bud (Table 1). In both years, the highest temperature level reduced the seeds produced per bud by 62 and 65 %, respectively compared with the low temperature level (Supplementary Data Table S3). Temperature also affected mean berry mass

TABLE 1. *Analyses of variance (ANOVAs) and G-tests examining differential response to temperature of two American ginseng populations*

	Overall survival[†]	RGR_{LA}[‡]	RGR_{ROOT}[§]	Bud number 2009	Bud number 2010	Seeds per bud 2009	Seeds per bud 2010	Berry mass per seed (2010)	Photosynthesis (PAR = 500) (2010)[¶]	Photosynthesis (PAR = 40) (2010)[¶]	Respiration 2010	Emergence (2009)	Pre-flowering buds: total buds (2009)	Pre-flowering buds: total buds (2010)	Senescence level	Root: Shoot
Temperature (T)	0	0.1	0.13	1.14	19·80***	4·04*	6·82**	4·12*	1·64	19·59***	16·89***	54·38***	50·79***	27·47***	47·83***	5·88***
Population (P)	0	1.09	7·58**	13·19***	1.47	11·67***	6·98***	0.03	0.09	6·57**	1.16	1.92	62·90***	0.17	0.03	0.45
T × P	0	2.45	0.24	2.5	3·74[13]	0.53	0.12	0.56	0.54	5·88**	0.55	2.41	0.26	3·97*	4·47**	4·61***

Test statistics are associated with main effects of temperature (T) and population (P), and interaction effects (T × P) by dependent variable for all statistical analyses. The test statistics provided are *F*-ratios, derived from two-way ANOVAs, unless otherwise specified. Statistically significant effects are highlighted with asterisks.

[†] The statistic listed is χ^2.
[‡] Relative growth rate of leaf area.
[§] Relative growth rate of root mass.
[¶] Photosynthetically active radiation.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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TABLE 2. *Response of two American ginseng populations to three temperature treatments*

	LowT Population			MedT Population		
	L	M	H	L	M	H
RGR_{LA} (cm^2 cm^{-2} $year^{-1}$)	1.01 ± 0.03	1.10 ± 0.03	0.89 ± 0.03	0.97 ± 0.03	0.97 ± 0.03	1.01 ± 0.03
RGR_{ROOT} (g g^{-1} $year^{-1}$)	0.64 ± 0.04	0.63 ± 0.04	0.65 ± 0.04	0.79 ± 0.05	0.86 ± 0.05	0.77 ± 0.05
Bud number 2009	17.08 ± 1.57	15.81 ± 1.50	10.76 ± 1.68	18.81 ± 2.29	21.73 ± 2.40	21.69 ± 2.19
Bud number 2010	26.85 ± 0.25	48.41 ± 0.23	57.02 ± 0.24	30.60 ± 0.31	47.06 ± 0.31	42.02 ± 0.31
Seeds per bud 2009	0.23 ± 0.03	0.18 ± 0.03	0.09 ± 0.03	0.10 ± 0.04	0.07 ± 0.04	0.03 ± 0.04
Seeds per bud 2010	0.25 ± 0.03	0.26 ± 0.03	0.12 ± 0.03	0.18 ± 0.04	0.16 ± 0.04	0.04 ± 0.04
Berry mass per seed (2010) (g)	0.41 ± 0.03	0.36 ± 0.03	0.21 ± 0.04	0.35 ± 0.04	0.40 ± 0.04	0.25 ± 0.10
Photosynthesis (PAR = 500) (2010) (mol CO_2 m^{-2} s^{-1})	2.84 ± 0.13	3.03 ± 0.13	2.76 ± 0.13	2.77 ± 0.13	3.01 ± 0.13	2.94 ± 0.13
Photosynthesis (PAR = 40) (2010) (mol CO_2 $m^{-2}s^{-1}$)	1.54 ± 0.04	1.41 ± 0.04	1.38 ± 0.04	1.50 ± 0.04	1.44 ± 0.04	1.11 ± 0.04
Respiration (2010) (mol CO_2 m^{-2} s^{-1})	-0.15 ± 0.02	-0.18 ± 0.02	-0.27 ± 0.02	-0.16 ± 0.02	-0.16 ± 0.02	-0.24 ± 0.02
Emergence level (1–6) (2009)	4.77 ± 0.14	5.75 ± 0.14	6.00 ± 0.14	4.20 ± 0.17	5.79 ± 0.18	6.00 ± 0.17
Pre-anthesis buds: total buds (2009)	0.55 ± 0.05	0.05 ± 0.05	0 ± 0.05	0.98 ± 0.07	0.48 ± 0.08	0.36 ± 0.07
Pre-anthesis buds: total buds (2010)	0.98 ± 0.04	0.66 ± 0.03	0.76 ± 0.03	1.00 ± 0.05	0.80 ± 0.05	0.66 ± 0.05
Senescence level (1–4) (2010)	1.00 ± 0.12	1.27 ± 0.12	2.55 ± 0.12	1.41 ± 0.16	1.18 ± 0.15	2.22 ± 0.15

Least squares means $\pm$ s.e. of dependent variables for interaction effects, listed by population (LowT Population, MedT Population) and temperature treatment (low, medium and high).
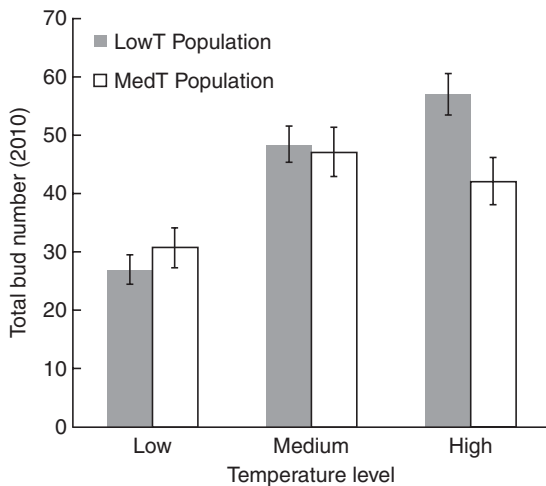

FIG. 1. Differential response of bud number between two populations of American ginseng to temperature. Ginseng populations originated from different temperature environments. The LowT Population is from a cooler, high elevation site, and the MedT Population is from a warmer, low elevation site. The low and medium temperature treatments correspond to the temperature regime at the LowT and MedT Populations, respectively, and the high temperature treatment simulates future temperature conditions.
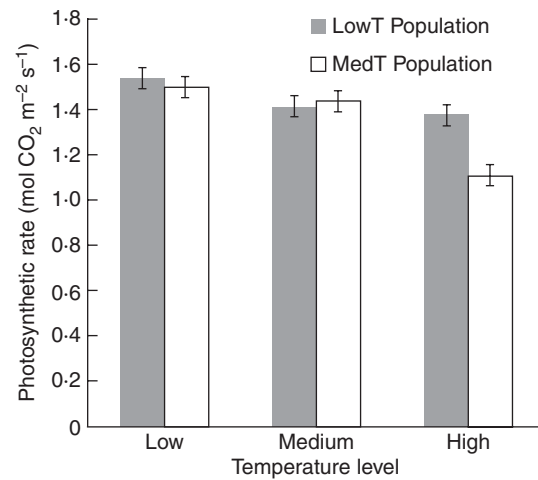

FIG. 2. Differential response to temperature of CO_2 assimilation rate between two populations of American ginseng. Ginseng populations originated from different temperature environments. The LowT Population is from a cooler, high elevation site, and the MedT Population is from a warmer, low elevation site. The low and medium temperature treatments correspond to the temperature regime at the LowT and MedT Populations, respectively, and the high temperature treatment simulates future temperature conditions.

per seed. Berry mass per seed was reduced by 41 % at the highest temperature level compared with the low temperature level. Populations differed in terms of seed production (Table 1). In both years of the study, the LowT Population produced a greater number of seeds per bud, LowT Population plants producing on average 2·58 and 1·64 times the number of seeds per bud relative to MedT Population plants in 2009 and 2010, respectively (Supplementary Data Table S3).

Physiological measurements

While there was no interaction effect of temperature and population on light-saturated photosynthetic rate, populations differed in their response to temperature at ambient growth light levels (Table 1). Populations responded differentially to the change in temperature between medium and high levels; photosynthetic rates decreased for plants from the MedT Population, but not for plants from the LowT Population (Fig. 2). In general, photosynthetic rates were highest in the low temperature treatment and lowest in the high temperature treatment (Table 2). Also, populations differed in terms of photosynthetic rates (Table 1). Plants from the LowT Population assimilated CO_2 at 1·07 times the rate of plants from the MedT Population (Supplementary Data Table S3). Temperature alone affected the respiration rate (Table 1). Respiration rates of plants in the high temperature treatment were 1·66 times greater than respiration rates of plants in the low temperature treatment.

Phenological measurements, physical appearance and allocation

The effect of temperature on timing of emergence in 2009 did not differ between populations (Table 1). The effect of temperature on bud phenology did not differ between populations in 2009, but did in 2010 (Table 1). Generally, increasing temperature accelerated onset of flowering, so that the ratio of pre-anthesis buds to total buds was lower in the medium and high temperature treatments relative to the low temperature treatment (Table 2). Plants from the LowT Population and the MedT Population responded differently to the transition from the medium and high temperature treatments. In the medium temperature treatment, MedT plants were phenologically behind LowT plants in their reproductive development, while the reverse was true in the high temperature treatment (Table 2). The effect of temperature on senescence level depended on population (Table 1). At the low temperature level, leaves of plants from the MedT Population showed greater levels of senescence than those from the LowT Population, while this effect was reversed at the high temperature treatment (Fig. 3). Higher temperatures advanced emergence, anthesis and senescence (Table 2). This temperature effect was, in some cases, profound. For example, in 2009, the proportion of pre-anthesis buds was reduced by 77 % in the high temperature level compared with plants in the low temperature level (Supplementary Data Table S3).

Plant biomass allocation of the two populations, as expressed by root to shoot ratio, differed in their response to temperature (Table 1). The mean root to shoot ratio of MedT Population plants did not vary among temperature treatments, while the root to shoot ratio of LowT Population plants did. Specifically, at the medium temperature treatment, the MedT Population plants allocated more biomass to root per unit mass of shoot than LowT Population plants, whereas the reverse was true at the high temperature treatment (Table 2).
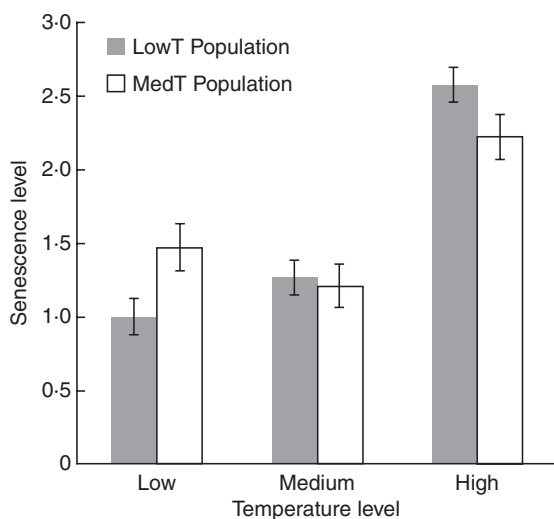

FIG. 3. Differential response of senescence level (on a scale of 1–4) to temperature between two populations of American ginseng. Ginseng populations originated from different temperature environments. The LowT Population is from a cooler, high elevation site, and the MedT Population is from a warmer, low elevation site. The low and medium temperature treatments correspond to the temperature regime at the LowT and MedT Populations, respectively, and the high temperature treatment simulates future temperature conditions.

DISCUSSION

A previous study examining the long-term demographic response of ginseng populations to interannual temperature variation found that population growth rates (λ) were greatest when growing season temperatures were similar to long-term, site-specific means (Souther and McGraw, 2011). When annual temperatures deviated from long-term mean conditions, λ values decreased, suggesting that populations were adapted with respect to local temperature regimes (Souther and McGraw, 2011). Based on these findings, we expected that populations would respond differently to temperature treatments, indicating genetic differentiation between populations. Secondly, we expected that traits probably predictive of long-term fitness, such as survival and seed production, or key physiological traits, such as photosynthesis, would be greater for plants exposed to their home site temperature environment relative to non-local individuals, indicating a direct effect of temperature on ginseng performance. The effect of temperature on bud number in 2010, photosynthetic rates, senescence level and root to shoot ratio differed between populations of ginseng, supporting the hypothesis that populations are genetically differentiated with respect to temperature. However, with the exception of one dependent variable (senescence level), the pattern of response did not conform to expectations that ginseng populations were adapted with respect to local temperature.

Temperature, alone, did not affect performance of ginseng populations in a manner consistent with local adaptation. Disparity between experimental results and those observed in the field may result, in part, because temperature variation was decoupled from variation in other environmental factors in the growth chamber environment. Abiotic environmental variables, such as soil moisture and light levels, as well as biotic factors, such as herbivory, prevalence of disease, and inter- and intraspecific competition interactions, naturally covary with temperature in the field and could influence demographic response to temperature (Hackney and McGraw, 2001; McGraw and Furedi, 2005; Wixted and McGraw, 2010). Previous reciprocal transplant and common garden studies have demonstrated that local adaptation may occur with respect to complex environmental gradients (Linhart and Grant, 1996; Macel *et al.*, 2007). Macel *et al.* (2007) partitioned the effect of climatic and edaphic factors on fitness-related traits and determined that both climate and soil were critical to understanding local adaptation in two species of grasses. In this case, mean growing season precipitation varied between the sites of origin of the study populations, the MedT population receiving approx. 20 % less rainfall on average than the LowT population in its home site. Response of stomatal conductance to temperature has been shown to depend on plant water status. Plants grown in an environment in which water is not limited respond to temperature by opening stomata, thus increasing transpiration rates and cooling the leaf. Conversely, plants exposed to drier conditions tend to close their stomata as temperature increases, reducing water loss through transpiration. (Berry and Bjorkman, 1980). Decreasing stomatal aperture reduces diffusion of CO_2 into plant cells, thus slowing photosynthetic rates (Berry and Bjorkman, 1980). Generally drier conditions at

the mid-elevation site may have selected for genotypes that decrease stomatal conductance in response to increasing temperature in order to prevent evaporative water loss from plant cells. This may, in part, explain the observation that MedT individuals displayed a greater reduction in photosynthetic rates relative to the LowT individuals in the high temperature treatment. Whatever the precise mechanism, for ginseng, indirect temperature effects may be more important determinants of population growth rate than the direct influence of temperature.

Lack of temperature 'extremes', temperatures that deviate significantly from mean conditions (in this case referring to deviations above the mean), in the growth chamber environment may also have contributed to disparity between field and growth chamber studies. While growth chamber temperatures cycled between realistic average minima and maxima on a daily basis, they did not include daily fluctuations of temperature around those means. Extreme temperatures constitute a strong selective force, and thus may drive patterns of local adaptation observed in the field (Parmesan *et al.*, 2000; Parmesan, 2006). In this study, individuals from the LowT Population were strongly affected by the high temperature treatment, which may more closely simulate extreme temperatures in the LowT environment. LowT individuals produced a greater number of buds relative to MedT plants at medium and high temperature treatments. Rather than indicating that LowT individuals are more successful than MedT individuals under high temperature conditions, results may also suggest a stress response, in which LowT individuals allocated more energy to reproduction when exposed to elevated temperatures. Increased flowering in response to environmental stress, and to temperature in particular, has been documented in other plant species (Bradshaw and Hardwick, 1989; reviewed in Takeno, 2012). The senescence response pattern further suggests that elevated temperatures induced a stress response in LowT individuals. Mean senescence level was lowest for each population in its home temperature treatment, consistent with expectations of local adaptation. Notably, however, senescence levels of LowT individuals increased dramatically in the high temperature treatment, and exceeded that of the MedT individuals. Senescence of leaves is triggered by a number of environmental stressors, including temperature (Buckner *et al.*, 1998; Munne-Bosch and Alegre, 2004). The degree of foliar senescence at the high temperature treatment far exceeded senescence levels observed in the field for this time of year (30 September 30, pers. obs.), and shortened growing season length for LowT individuals exposed to high temperature conditions.

This study joins a growing body of literature demonstrating intraspecific genetic variation to temperature, and documenting the effects of warming on components of plant fitness (Etterson, 2004; Macel *et al.*, 2007; De Frenne *et al.*, 2011, 2012). Intraspecific genetic variation will influence the nature of species' response to climate change, as well as a species' adaptive potential (Davis and Shaw, 2001; Etterson, 2004; Davis *et al.*, 2005; Jump and Penuelas, 2005). In this study, temperature treatments alone did not explain the pattern of local adaptation to temperature observed in the field. For ginseng, increases in the frequency and magnitude of extreme weather events, as well as the indirect effects of climate warming, will perhaps be more important than the direct effects of mean temperature increase in determining response to climate change.

Genetic differentiation of populations with respect to temperature has implications for species management and conservation as climate change occurs. Ginseng harvest, regulated by the US Fish and Wildlife Service, should be managed to minimize loss of genetic diversity, thus enhancing the potential for adaptation to increasing temperature. In general, for species that exhibit intraspecific differentiation to climate, rather than protecting northern or upland populations, effort should be focused towards preservation of genetically diverse populations, which have greater potential to adapt to novel climatic conditions, and towards the establishment of dispersal corridors that may encourage colonization of cooler regions in response to warming. When a species is unable to track climate change naturally through genetic or spatial response, human-assisted relocation may be necessary for long-term persistence.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Table S1: daily minimum and maximum temperatures used to program growth chambers. Table S2: analyses of variance and *G*-tests examining differential response to temperature of the two populations. Table S3: response of the two populations to three temperature treatments.

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LITERATURE CITED

Anderson MP. 1996. *American ginseng (Panax quinquefolium L.) in south-western Wisconsin: patterns of demography and habitat associations.* MSc thesis, University of Wisconsin, Madison, WI, USA.

Anderson RC, Fralish JS, Armstrong JE, Benjamin PK. 1993. The ecology and biology of *Panax quinquefolium* L. (*Araliaceae*) in Illinois. *American Midland Naturalist* **129**: 357–372.

Antonovics J. 2006. Evolution in closely adjacent plant populations X: long-term persistence of prereproductive isolation at a mine boundary. *Heredity* **97**: 33–7.

Bailey B. 1999. *Social and economic impacts of wild harvested products.* PhD thesis, West Virginia University, Morgantown, WV, USA.

Bazzaz FA, Williams WE. 1991. Atmospheric CO_2 concentrations within a mixed forest: implications for seedling growth. *Ecology* **72**: 12–16.

Berry J, Bjorkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **31**: 491–543.

Bradshaw AD, Hardwick K. 1989. Evolution and stress—genotypic and phenotypic components. *Biological Journal of the Linnean Society* **37**: 137–155.

Buckner B, Janick-Buckner D, Gray J, Johal GS. 1998. Cell-death mechanisms in maize. *Trends in Plant Science* **3**: 218–223.

Charron D, Gagnon D. 1991. The demography of northern populations of *Panax quinquefolium* (American ginseng). *Journal of Ecology* **79**: 431–445.

Cruse-Sanders JM, Hamrick JL. 2004. Genetic diversity in harvested and protected populations of wild American ginseng, *Panax quinquefolius* L. (*Araliaceae*). *American Journal of Botany* **91**: 540–548.

Davis MB, Shaw RG. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science* **292**: 673–679.

Davis MB, Shaw RG, Etterson JR. 2005. Evolutionary responses to changing climate. *Ecology* **86**: 1704–1714.

De Frenne P, Brunet J, Shevtsova A, et al. 2011. Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Global Change Biology* **17**: 3240–3253.

De Frenne P, Brunet J, Shevtsova A, et al. 2012. The response of forest plant regeneration to temperature variation along a latitudinal gradient. *Annals of Botany* **109**: 1037–1046.

Etterson JR. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. 1. Clinal patterns of selection along an environmental gradient in the great plains. *Evolution* **58**: 1446–1458.

Futuyma DJ, Moreno G. 1988. The evolution of ecological specialization. *Annual Review of Ecology and Systematics* **19**: 207–233.

Grubbs HJ, Case MA. 2004. Allozyme variation in American ginseng (*Panax quinquefolius* L.): variation, breeding system, and implications for current conservation practice. *Conservation Genetics* **5**: 13–23.

Hackney EE. 1999. *The effect of small population size, breeding system, and gene flow on fruit and seed production in American ginseng (Panax quinquefolius L., Araliaceae)*. Msc thesis, West Virginia University, Morgantown, WV, USA.

Hackney EE, McGraw JB. 2001. Experimental demonstration of an Allee effect in American ginseng. *Conservation Biology* **15**: 129–136.

Holt RD. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences, USA* **106**: 19659–19665.

Holt RD, Barfield M. 2008. Habitat selection and niche conservatism. *Israel Journal of Ecology and Evolution* **54**: 295–309.

Holt RD, Gaines MS. 1992. Analysis of adaptation in heterogeneous landscapes: implications for the evolution of fundamental niches. *Evolutionary Ecology* **6**: 433–447.

IPCC. 2007. *Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press.

Jochum GM, Mudge KW, Thomas RB. 2007. Elevated temperatures increase leaf senescence and root secondary metabolite concentrations in the understory herb *Panax quinquefolius* (*Araliaceae*). *American Journal of Botany* **94**: 819–826.

Jump AS, Penuelas J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters* **8**: 1010–1020.

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecology Letters* **7**: 1225–1241.

Kota NL. 2005. *Comparative seed dispersal, seedling establishment and growth of exotic, invasive Ailanthus altissima (Mill.) Swingle and native Liriodendron tulipifera (L.)*. MSc thesis, West Virginia University, Morgantown, WV, USA.

Lewis WH, Zenger VE. 1983. Breeding systems and fecundity in the American ginseng, *Panax quinquefolium* (*Araliaceae*). *American Journal of Botany* **70**: 466–468.

Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* **27**: 237–277.

Macel M, Lawson CS, Mortimer SR, et al. 2007. Climate vs. soil factors in local adaptation of two common plant species. *Ecology* **88**: 424–433.

Matesanz S, Gianoli E, Valladares F. 2010. Global change and the evolution of phenotypic plasticity in plants. *Year in Evolutionary Biology* **1206**: 35–55.

McGraw JB, Furedi MA. 2005. Deer browsing and population viability of a forest understory plant. *Science* **307**: 920–922.

McGraw JB, Sanders SM, Van der Voort M. 2003. Distribution and abundance of *Hydrastis canadensis* L. (*Ranunculaceae*) and *Panax quinquefolius* L. (*Araliaceae*) in the central Appalachian region. *Journal of the Torrey Botanical Society* **130**: 62–69.

Mooney EH, McGraw JB. 2009. Relationship between age, size, and reproduction in populations of American ginseng, *Panax quinquefolius* (*Araliaceae*), across a range of harvest pressures. *Ecoscience* **16**: 84–94.

Munne-Bosche S, Alegre L. 2004. Die and let live: leaf senescence contributes to survival under drought stress. *Functional Plant Biology* **31**: 203–216.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics* **37**: 637–669.

Parmesan C, Root TL, Willig MR. 2000. Impacts of extreme weather and climate on terrestrial biota. *Bulletin of the American Meteorological Society* **81**: 443–450.

Riis T, Lambertini C, Olesen B, Clayton JS, Brix H, Sorrell BK. 2010. Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation? *Annals of Botany* **106**: 813–822.

Robbins CS. 2000. Comparative analysis of management regimes and medicinal plant trade monitoring mechanisms for American ginseng and goldenseal. *Conservation Biology* **14**: 1422–1434.

Savolainen O, Pyhajarvi T, Knurr T. 2007. Gene flow and local adaptation in trees. *Annual Review of Ecology Evolution and Systematics* **38**: 595–619.

Schlessman MA. 1985. Floral biology of American ginseng (*Panax quinquefolium*). *Bulletin of the Torrey Botanical Club* **112**: 129–133.

Souther S, McGraw JB. 2010. Vulnerability of wild American ginseng to an extreme early spring temperature fluctuation. *Population Ecology* **53**: 119–129.

Souther S, McGraw JB. 2011. Evidence of local adaptation in the demographic response of American ginseng to interannual temperature variation. *Conservation Biology* **25**: 922–931.

Takeno K. 2012. Stress-induced flowering. In: Ahmad P, Prasad MNV. eds. *Abiotic stress responses in plants: metabolism, productivity, and sustainability.* Springer: New York, 331–346.

Van der Voort M. 2005. *An ecological study of Panax quinquefolius in central Appalachia: seedling growth, harvest impacts and geographic variation in demography.* PhD thesis, West Virginia University, Morgantown, WV, USA.

Ward D, Shrestha MK, Golan-Goldhirsh A. 2012. Evolution and ecology meet molecular genetics: adaptive phenotypic plasticity in two isolated Negev desert populations of *Acacia raddiana* at either end of a rainfall gradient. *Annals of Botany* **109**: 247–255.

Wixted KL, McGraw JB. 2010. Competitive and allelopathic effects of garlic mustard (*Alliaria petiolata*) on American ginseng (*Panax quinquefolius*). *Plant Ecology* **208**: 347–357.