Original article

Natural selection on plant physiological traits in an urban environment

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ABSTRACT

Current rates of urbanization are creating new opportunities for studying urban plant ecology, but our knowledge of urban plant physiology lags behind that of other ecosystems. Moreover, higher temperatures, elevated CO_2, and increased inorganic nitrogen deposition along with altered moisture regimes of urban as compared to rural areas creates a compelling analog for studying adaptations of plants to climate change. We grew plants under common conditions in a greenhouse to determine whether populations of *Crepis sancta* (Asteraceae) differed in phenological, morphological, and physiological traits. We also used a field experiment to test for natural selection on these traits in urban Montpellier, France. Urban plants flowered and senesced later than rural plants, and natural selection favored later phenology in the urban habitat. Natural selection also favored larger plants with more leaves, and increased photosynthesis and leaf nitrogen concentration. Ours is the first study to document selection on plant functional traits in an urban habitat and, as such, advances our understanding of urban plant ecology and possible adaptations to climate change.

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1. Introduction

The rapid expansion of urban boundaries is increasing the number of organisms that live in contact with cities. Although this contact has significant implications for plant growth and function, our understanding of urban plant ecology has trailed behind that of other ecosystems (Grimm et al., 2008; Wang and Pataki, 2010). Abiotic conditions of urban environments are markedly different from those of adjacent rural areas. Urban environments typically experience higher CO_2 levels, elevated temperatures, and increased deposition of inorganic nitrogen pollutants, as compared with rural environments (Gregg et al., 2003; Carreiro and Tripler, 2005; Pataki et al., 2006; Grimm et al., 2008; Searle et al., 2012). The elevated temperatures of cities are particularly pronounced at night, due to urban "heat islands" created by cement and other building materials radiating heat that was absorbed during the day (Arnfield, 2003). While increases in CO_2, nitrogen, and temperature will generally enhance photosynthesis, warmer temperatures will also increase rates of respiration and of soil water evaporation, which can lower net photosynthetic carbon gain. Moreover, interactions among these abiotic factors can elicit even more complex responses. In addition to the importance of studying urban plant ecology, some of these altered abiotic conditions of the urban environment are similar to those we expect in the future as climate change progresses (Carreiro and Tripler, 2005). By using the urban environment as an analog of climate change, we can quantify how plants have adapted to an altered climate and, therefore, improve our ability to make predictions about adaptations to climate change in the future.

There have been a few investigations of the growth and physiological response of plants to urban climates, but their focus has been on plasticity and acclimation. Studies that have examined the acclimation of growth and physiological rates of young trees planted along an urban-rural gradient from New York City to surrounding areas have found that plants grew larger and faster in the urban habitats, including having greater biomass, increased leaf area, and an increased number of leaves (Gregg et al., 2003; Searle et al., 2012). Although plasticity is undoubtedly an important mechanism allowing plants to acclimate to a changing climate, natural selection must also be shaping the plant functional response to both urban climates and to climate change. However, there are no studies of natural selection on plant physiological traits in urban environments.

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In order to study plant evolution in response to an urban climate, we used the recent colonization of a native annual weed, *Crepis sancta,* to urban environments in southern France. In urban areas, *C. sancta* grows in sidewalk cracks, around planted trees, and in disturbed lots, while in rural areas, it is found in vineyards and along roadsides. Plants produce both heavy, non-dispersing seeds along with seeds with a dispersing pappus (Cheptou et al., 2008). Previous research has documented rapid evolution toward reduced seed dispersal in *C. sancta* in the urban environment, presumably because the urban concrete matrix selects against dispersal (Cheptou et al., 2008).

Given that *C. sancta* has demonstrated evolution in dispersal traits in response to the urban environment, we used this species to study its evolution in growth and physiological traits. In this study, we tested whether there is evidence for natural selection on physiological traits in an urban habitat. To assess selection, we transplanted plants grown from seeds collected from on urban and one rural population to an urban area in Montpellier, France. We measured phenological, morphological, and physiological traits, and quantified natural selection by looking at the relationship between fitness and these traits. We also describe differences observed between plants from the same two populations grown together in a greenhouse. Our previous work with these populations has documented that each is representative of their respective environment for several phenological and morphological traits (Cheptou et al., 2008; Dubois and Cheptou, unpublished).

2. Methods

2.1. Study species and field populations

Crepis sancta L. (Bornm.) (Asteraceae) is a winter annual that is native to the Mediterranean region. Its seeds germinate in the fall, with the onset of rains, and it overwinters as a rosette. In spring (~Mar–May), it elongates flowering stalks on which it bears capitula. Following seed set and dispersal, the plants senesce.

Our experiments included plants originating from two populations used in our previous studies of *C. sancta* (Cheptou et al., 2002, 2008). The rural population was a large unfragmented population located in a vineyard in Claret, France (43° 51.13′N 3° 53.71′E). The urban population was located ~30 km away along a street in Montpellier (43° 36.42′N 3° 53.54′E) where plants were grew in patches around the base of cultivated street trees. The urban area was developed in 1982, before which it was agricultural.

During the 2012–13 growing season, temperature and soil moisture data were collected in each population to characterize differences. Four iButton temperature loggers (Maxim Integrated, San Jose, CA, USA) were placed at leaf height in each population to record temperature hourly from March–May 2013, which coincides with the growing season of *C. sancta*. Weather data were also obtained from nearby (<5 km) meteorological stations (Association Climatologique de l'Hérault, Montpellier, France) from July 2012–June 2013. Additionally, to determine gravimetric soil water content, five soil samples from the upper 15 cm of soil were collected every 2–3 weeks from February–May (total of 7 collections). Samples were placed in airtight containers and transported to the lab in a cooler. After initial weights were recorded, samples were dried in an oven at 60 °C for 72 h, and then reweighed. The difference was used to calculate percent water content. The iButton temperatures and the soil moisture data were compared between populations using repeated measures general linear model in SPSS v. 22.0 (IBM Corporation, Armonk, NY, USA).

2.2. Natural selection in the urban environment

In November 2012, seeds collected from each of the populations were sown in a naturally lit greenhouse at the "Plateforme des Terrains d'Experience du LabEx CeMEB". Seeds were collected from ~35 randomly selected maternal plants in each population. Previous genetic studies with these same populations revealed low levels of relatedness between plants, even those relatively close to one another (Cheptou et al., 2002). Collected seeds were sown into 0.7 L rhizotrons filled with a 1:1:1 ratio of sand, humus, and field soil. Plants were watered every 2–3 d to maintain non-limiting soil moisture. Ambient photoperiod, with ~10 h day length, was used. On 11 December 2012, seedlings had ~4 true leaves and were transplanted to ~1–2 m^2 patches around the base of trees along a street in Montpellier, France. Patches were cleared of other herbaceous vegetation prior to transplantation. Each of the 50 patches was randomly assigned plants from only one of the populations, with ~10–20 plants per patch. During March and April 2013, patches and plants were randomly selected for measurement, with 1–2 plants measured per patch.

2.2.1. Phenological and morphological traits

Parameters of life-history and size were measured for 120 randomly selected plants (60 originating from each population). Date of senescence was recorded to assess phenology. Capitula number was counted on the date of senescence to estimate fitness. The number of leaves (>2 cm length) and the diameter of the rosette, measured in two perpendicular measurements to the nearest 0.5 cm, were recorded on the day the first capitula opened. Then, the most recent, fully expanded leaf was collected and a digital photograph was taken, including a ruler for scale. Leaf area was determined using ImageJ v. 1.6 (Schneider et al., 2012). These leaves were dried in an oven at 60 °C for 72 h. Dry weights were measured and specific leaf area (SLA) was calculated as the ratio of leaf area to leaf mass. Dried leaves were ground and used for analyses of δ^{13}C and leaf nitrogen content (see section on "Physiological traits").

2.2.2. Physiological traits

Instantaneous rates of photosynthesis (A) and stomatal conductance (g) were measured using a LiCor 6400 (Licor Biosciences, Lincoln, NE, USA) on 30 plants per population (N = 60), using the same leaf collected for area and δ^{13}C analyses. All measurements were made between 0900 and 1200 h CET. During measurements, conditions in the cuvette were controlled so that photosynthetic photon flux density was held at saturating 1000 μmol m^{-2} s^{-1}, leaf temperature was 20 °C, and [CO$_2$] was 38 Pa. Vapor pressure deficit during measurements was 0.67 ± 0.02 kPa. All measures were area-corrected, using digital photos and Image J analysis of leaf area for those leaves that did not fill the cuvette. Instantaneous water-use efficiency was calculated as the ratio between photosynthesis and stomatal conductance.

Collected leaf samples were used to determine leaf nitrogen content ([N]) and integrative water-use efficiency, as inferred from stable carbon isotope ratios (δ^{13}C). Carbon isotope ratios provide a time-integrated estimate of water-use efficiency, or the ratio of carbon assimilated to water lost via stomatal conductance. Higher values (less negative) indicate higher water-use efficiency, when compared to plants grown under similar [CO$_2$] and water vapor conditions (Dawson et al., 2002). A 4.0 mg (±0.2 mg) subsample of ground material was analyzed for δ^{13}C and [N] using a Delta-V Advantage isotope ratio mass spectrometer (Thermo-Electron, Bremen, Germany) interfaced with an Euro EA elemental analyzer (Eurovector, Milan, Italy) at the Fractionnement des AgroResources et Environnement (INRA, Reims, France). Carbon isotope ratios of