Latitudinal effects on phenological and reproductive traits in the invasive *Impatiens glandulifera*[1]

In the present study, various traits related to the phenology (timing of events) and the reproduction of the invasive *Impatiens glandulifera* (Balsaminaceae) have been recorded on individuals originating from populations situated along a 1600 km latitudinal gradient. Individuals have been grown for two consecutive generations in a common environment in a greenhouse. A brief description of the species and the study design is presented below. Analyze these data. You will pay particular attention to explain how the statistical tests you perform correspond to the hypotheses you want to test. Present clearly the results with tables and figures. Provide a rapid discussion.

Study species

Impatiens glandulifera Royle (Balsaminaceae), the Himalayan balsam is native to western Himalaya – northern Indian provinces from Kashmir to Garhwal and in Pakistan where it grows along roadside ditches and around field borders between 2000 – 4000 m a.s.l. It was introduced from Kashmir into England as an ornamental garden plant in 1939, but recorded presence of the species in some botanical gardens in Germany in 1832 and recent molecular work strongly suggest multiple introductions to Europe. By 1982, it had spread all the way to northern England and later across Europe via a combination of introductions into botanic and private gardens and natural spread facilitated by transport along rivers. With a maximum height of 3 m, *I. glandulifera* is one of the tallest annual plants in Europe. The species produces protandrous flowers from July to October, setting seeds from mid-July onwards. Flower colour varies from dark purple to pink and white.

Methods

The populations used in this study were sampled in six different regions distributed along an ca. 1600 km latitudinal transect in Western Europe (from Amiens, France 49.91°N to

[1] Data taken from the Thesis of Kamal Prasad Acharya

Trondheim, Norway 63.48°N) (Fig. 1). In each region, seeds from 30 individuals were sampled from one population. We collected all matured capsules from each individual. The collected seeds were stored in paper bag at room temperature. Populations were sampled in relatively similar habitat.

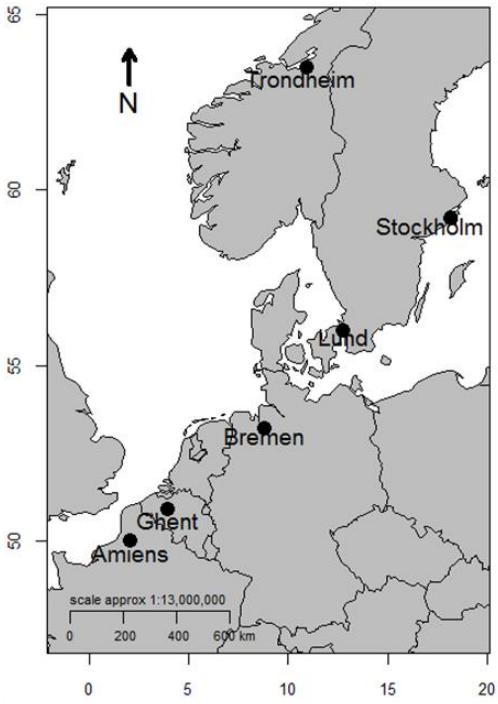

Figure 1: Location of the six population of *I. glandulifera* sampled in this study.

In November 2011, 30 seeds per maternal plant were stratified at 4°C for two months on a moist filter paper in 90 mm diameter Petri dishes tightened with parafilm to maintain moisture. After germination, in the end of January 2012, five seedlings from each maternal plant were planted in a plastic pot (12 cm diameter, 15 cm height) in a greenhouse in Trondheim, Norway (63.4°N). The plastic pots were filled with about 1.5 litres of soil. Upon reaching the true two-leafed stage, seedlings were thinned to one individual per pot, giving a total of 30 individual plants per population (total 180 individuals). Individuals from each population were haphazardly placed on eight trays in two rooms (four trays in each room) in the greenhouse. For the first month, additional light was provided with a 12:12 LD cycle. The average temperature was 10°C during the day and 4°C at night. After successful establishment (ca. 15 cm tall), the plants were grown at an average temperature of 18°C during the day and 8°C at night.

Plants were fertilized once a week. To minimize the position effect and the room effect on the phenotype of the plants, individuals were randomly moved within a room once every week and between rooms every two weeks as long as it was possible to move them (ca. 1 m height). When individuals had produced at least 10 flowers, we crossed them with other individuals from the same population by hand pollination. Pollen donors and receivers were randomly assigned within each population. Hand pollination was carried out by removing matured anthers from a flower of the pollen donor and applying the pollen to receptive stigmas of a newly opened flower of the maternal individual. In order to prevent self-pollination, the anther from the pollinated flower was also removed. Hand-pollinated flowers were marked with a strip of adhesive tape around the flower stalk. Some of the pollinated capsules died and we conducted new pollinations to ensure that at least 10 matured capsules could be harvested

per individual. Before the capsules matured, light tea bags (9.5 cm × 14 cm) were placed around the capsules to collect seeds. These seeds were used to produce the second greenhouse generation that was raised from January to June 2013 with the same procedure.

Trait measurements

We recorded the onset of flowering as the time (in days) between germination and the opening of the first flower. At the end of the growing season, we measured plant height (in cm) from the soil surface to the uppermost photosynthetic tissues (excluding inflorescences) (cf. Cornelissen *et al.* 2003) and we counted the total number of flowers produced by each individual. For the flowers that had already fallen off we counted the scars in the inflorescence to estimate the total number of flowers produced. We collected a minimum of 10 matured capsules obtained through artificial pollination per plant in a tea bags and harvested the above ground parts. Collected seeds were air-dried for 8 weeks at room temperature and weighted to obtain mean seed mass per plant. The collected seeds were used to calculate the average number of seeds per capsule for each plant individual by dividing the total number of seeds collected by the total number of capsules harvested for that plant. The aboveground biomass was harvested and oven-dried at 60°C for 72 hours and subsequently weighted.

Literature

Olson K, Ågren J. 2002. Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb *Lythrum salicaria*. *Journal of Evolutionary Biology* 15: 983-996.

Kollmann, J, Banuelos MJ. 2004. Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions* 10: 377-385.