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Some attempts to detect genetic differences between populations of small balsam (*Impatiens parviflora* DC.)

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Abstract: Invasive plants populations often originate from a few individuals, thus they may show very low genetic variability. On the other hand, differences between local populations can result from a rapid evolutionary change, which may play important role in the invasion process. We attempt to detect genetic differences between two Polish populations of small balsam, an annual plant highly invasive in Europe. Studies are conducted in parallel at two levels: morphological (by common garden experiments) and molecular (using AFLP method). AFLP shows no differences between the two populations, but the results obtained for some morphological measurements suggest that slight genetic differences between the two populations exist. Possibly they are too small to be detected through AFLP technique. Some of them can result from maternal effects; this is to be checked in further research.

Key words: genetic variability, *Impatiens parviflora*, small balsam, common garden experiment, interpopulation variability, morphological measurements, AFLP, invasion

1. Introduction

The success of invasive alien plants is often ascribed to their high phenotypic plasticity. At the same time, populations of invasive plants, due to founder effect and relatively short time from introduction, are expected to show a low level of genetic variability (Williamson 1996; Sultan 2000). During last years, several reports provided new evidence about rapid evolutionary change of invasive species. Such a change may result in genetic differentiation between local populations and play important role in the invasion process (Lee 2002; Parker *et al*. 2003; Kollmann & Bañuelos 2004).

Impatiens parviflora, which originated from Central Asia, is an annual species highly invasive in almost whole Europe and appears in various habitats. Our research attempts to detect genetic differences between populations of small balsam, which grow in considerable geographical distances and in extremely different habitat conditions.

2. Material and methods

Our study has been focused so far on two Polish populations of small balsam, which inhabit locations differing in habitat conditions: (*i*) floodplain ash-elm forest population from the „Las Bielański" nature reserve in Warsaw, hereafter denoted by R; (*ii*) railroad track population from Nowosady, near Hajnówka (N-E Poland), hereafter denoted by T. In order to detect genetic differences between these populations we used two parallel approaches.

2.1. Experiments in uniform conditions (common garden experiments)

Seeds were collected in the summer 2004 in respective populations. In 2005 we used them for two common garden experiments conducted in the glasshouse: (*i*) experiment in competition conditions, hereafter referred to as the population experiment. Plants were grown in rockwool, watered periodically with

Kemira® solution, at two density levels (about 50 m^2 and about 300 m^2); (*ii*) experiment on individually grown plants, hereafter referred to as the pot experiment. Plants from both populations were grown in separate pots and two variants of growth conditions were arranged, differing in soil used and its moisture, irradiance level and air humidity – in this way differences between conditions encountered in R and T habitats were simulated to a degree. Seed germination and seedling emergence was traced in detail during the pot experiment. In both experiments we measured several plant traits, among them: stem height and diameter, number of generative shoots and number of flowers. At harvest, aboveground parts were divided into stems, leaves and generative parts and their dry mass was determined separately for each plant. In the case of pot experiment fresh mass was also recorded at harvest. In total, nearly 500 plants were grown in the population experiment, and about 250 plants in the pot experiment.

2.2. AFLP analysis of DNA

Healthy leaves collected in summer 2004 were dried and stored in silica gel. The AFLP analysis followed the procedure by Vos *et al.* (1995) with minor modifications (Bednarek *et al.* 1999). 22 individuals from each population were analysed. A pre-amplification step was performed in the presence of primers with one selective nucleotide. For the selective amplification four primer combinations: *Eco*RI-AGG/*Mse*I-CCC, *Eco*RI-AAA/*Mse*I-CAC, *Eco*RI-AGG/*Mse*I-CCA, *Eco*RI-AGT/*Mse*I-CTT with two additional nucleotides at the 3'-ends were used. The *Eco*RI compatible primers were labelled at their 5'-ends with gamma - ^{32}P ATP. PCR products were separated on 5% PAGE and exposed to X-ray film at –70°C overnight. PCA and AMOVA were used for data analyses.

3. Results

Germination speed and growth of seedlings. Mean germination time of seeds from the R population was about two days shorter than that of seeds from the T population (Fig. 1, ANOVA, $p<0.0001$, number of R plants $N_R=128$, number of T plants $N_T=120$); there was also respective difference in the time of seedling emergence. Individually growing plants derived from R seeds were higher during the first period of growth, both in T and R conditions.

Water content. Under high density conditions in the population experiment, R plants had higher aboveground dry mass than T plants when controlling for plant height and stem diameter (ANCOVA, $p=0.002$). This suggested their lower relative water content (not examined on that occasion). Indeed, in the pot experiment, T plants were more hydrated than R plants under T conditions (Fig. 2).

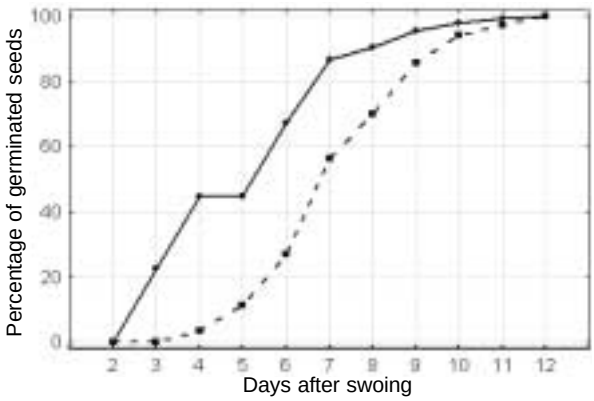

Fig. 1. Time of seed germination for plants from R (solid line) and T (dotted line) source populations
Explanation: Percentage of germinated seeds is presented as relative to the total number of seeds germinated after 12 days

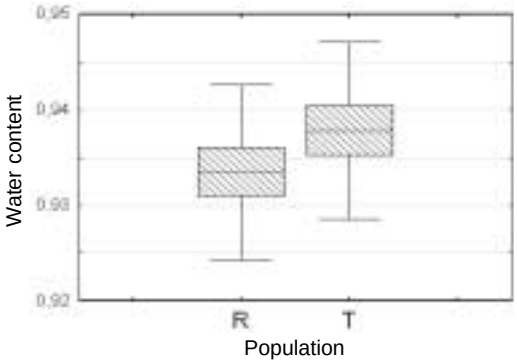

Fig. 2. Relative water content in aboveground fresh mass of plants in the pot experiment, T conditions
Explanations: solid line inside the box – mean; box – 95% confidence limits for mean; whiskers – standard deviation

Reproductive effort. The proportion of generative parts in aboveground dry mass of plants was higher for R than for T plants in the population experiment, at both densities (Fig. 3). Consistently, the number of generative shoots and flowers was higher at harvest

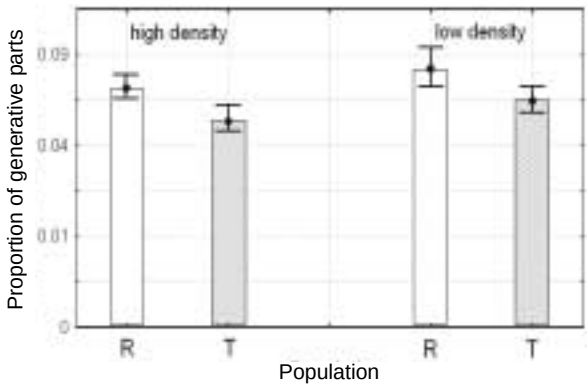

Fig. 3. Proportion of generative parts in aboveground dry mass of plants from R and T populations, in both densities in the population experiment
Explanations: dot – mean; whiskers – 95% confidence limits for mean

for R than for T plants in the pot experiment under T conditions (Fig. 4).

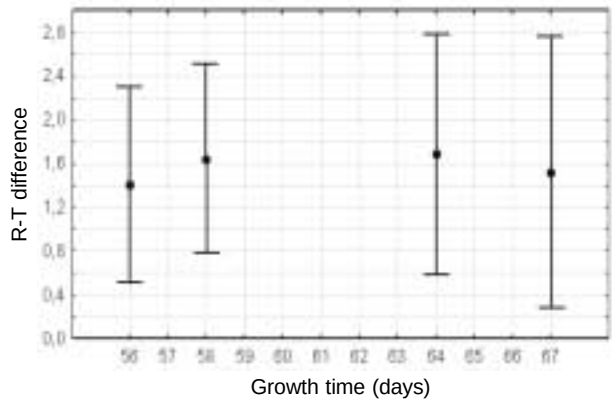

Fig. 4. Difference in number of generative shoots between R and T plants grown in T conditions in the pot experiment
Explanations: dot – difference of means, whiskers – 95% confidence limits for the difference of means

AFLP analysis of genetic variability. In total, all primer pairs combinations amplified 236 bands that were generated for all DNA templates isolated and processed according to the AFLP approach from plants collected from both localities. The number of the DNA fragments generated by individual primer pair varied from 50 to 72 with an average of 59. First three from four primer pairs listed above generated polymorphic signals and only 6% of the identified DNA fragments exhibited polymorphisms. AMOVA and PCA gave no evidence for differences between the two populations.

4. Conclusions

The results of garden experiments suggest that genetic differences exist between the two small balsam populations studied. Some observed differences may result from maternal effects and we will check for this in further research, using next generation of plants obtained in uniform conditions. On the other hand, in view of the contrast between habitats, differences in relative water content can be considered a result of locally adaptive changes in water economy arising from evolutionary changes over short time. Therefore, we have turned to anatomical traits related to water economy.

Variability between the two populations may be still too small to be detected by AFLP method. In subsequent studies we will consider populations more distant geographically.

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