Ecography

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Montesinos, D. and Callaway, R. M. 2016. Inter-regional hybrids of native and invasive *Centaurea solstitialis* display intermediate competitive ability. – Ecography doi: 10.1111/ecog.02653

Supplementary material

Appendix 1

Material and methods

Centaurea solstitialis is an annual herb with a self-incompatible reproductive system (Sun and Ritland 1998), which greatly facilitates the experimental production of seeds of known ancestry via manual crosses. The species was introduced into California at the latest in 1824 (Madd*ox et al*, 1985) where it has become an aggressive invader. Genetic data suggests that reintroductions into California have been frequent in the past (Dlugosch et al. 2013, Eriksen et al. 2014), but the absence of information about new introductions suggests that new reintroductions are unlikely (DiTomaso et al. 2006). Inter-specific hybridization is believed to provide with adaptive advantages to invasive species in the form of (1) evolutionary novelty; (2) increased genetic variation; (3) fixed heterosis; and (4) dumping genetic load, which typically result in enhanced vigor and invasive ability (Ellstrand and Schierenbeck 2000, Schierenbeck and Ellstrand 2008). However genetic studies found no traces of inter-specific hybridization on *C. solstitialis* (Garcia-Jacas et al. 2006, Dlugosch et al. 2013, Eriksen et al. 2014).

During the summer of 2009 we collected seeds from fifteen different individuals from ten different populations each across Spain and California (see Montesinos et al. 2012). Although the sampling was reasonably good, biogeographic studies of this kind can never completely discard that the detected differences between regions are not the result of founder effects (Bossdorf et al. 2005). Also, we can not differentiate if the detected patters would be the result of adaptive or non-adaptive evolutionary changes (Keller and Taylor 2008, Lachmuth et al. 2011) although this would not affect our conclusions. Two factors suggest that founder effects are unlikely to be important for our study: firstly, several biogeographic studies show a very similar pattern of increased competitive ability in Californian populations of *C. solstitialis* (Widmer et al. 2007, Graebner et al. 2012, García et al. 2013); secondly, regardless of potential bottleneck effects, the experimental design would still illustrate how increased competitive ability is inherited when populations of the same invasive species with significantly different levels of competitive ability are experimentally crossed.

In 2010, seeds from each individual were grown in common conditions in a greenhouse from which pollinators were excluded. Seeds were germinated and grown in pots, and each individual adult plant was subjected to a set of different manual pollination treatments. These included random cross-pollinations with other individuals from a different population within the same region, and with individuals from a different region (see detailed methodology in Montesinos et al. 2012). This produced four groups of seeds based on the geographic origin of their parents: seeds with a Californian mother and father; a Californian mother and a Spanish father; a Spanish mother and a Californian father; or a Spanish mother and father.

In 2011 we used these four kind of seeds (hereafter “treatments”) to set up a common garden competition experiment in which seeds from each of the four treatments were grown either alone in control pots, or in competition with one individual *Bromus hordeaceus*. This European native annual grass has replaced Californian native grasses over large areas and thus has overlapping distributions with *C. solstitialis* both in their native range of Spain and in their invasive range of California (Hastings and Ditomaso 1996). Field-collected *Bromus hordeaceus* seeds from California were purchased from S&S Seeds, Carpinteria, CA, USA. We used *C. solstitialis* F1 sibling individuals from the same specific father and mother for each replication between paired treatments (i.e. control and competition pots).

The number competitor species to be used in our experiment was constrained by the limited number of inter-regional hybrid seed available, which made the choice of one single and well-known strong competitor species a need. Based on previous competition studies (Graebner et al. 2012) we chose *B. hordeaceus* as a strong competitor sharing the same European native range and American non-native range that *C. solstitialis*. Other studied native grasses were weak competitors and did not trigger sufficient competitive responses in *C. solstitialis*, and thus were deemed unsuitable for tests of biogeographical differences in competitive ability of *C. solstitialis*. Additionally, it would have been desirable to compare competition ability from *B. hordeaceus* seed from both native and non-native ranges; however, the complexity of such an experimental design and the limited availability of inter-regional hybrid seed made it unfeasible.

Sibling *C. solstitialis* seeds were planted either in control pots or in competition with *B. hordeaceus* in 200 mL Ray Leach Inc. *Cone-Trainers* pots in a 50:50 mix of 20-30 grit sand and soil from Missoula, MT, and watered every 1-2 days. Each treatment had ten control and ten competition replicates, with inter-regional hybrids being the result of crosses between random pairs of individuals from different pairs of native and non-native populations. A control group of *B. hordeaceus* was also sown (N=30). We fertilized each pot with 100 mL of 1.16 g L^{-1} Scotts Miracle-Gro (15 N:30 K:15 P + micronutrients) once, four weeks after germination. Plants were grown for 57 days, at which time their rosettes had reached roughly maximum sizes but growth of flowering stems had not started. Total shoot and root biomass was harvested and dried at 70^{o}C for 48h and then weighed. There was no mortality in the experiment.

We used data for total dry biomass of *C. solstitialis* plants from each seed ancestry growing either in control pots or in competition with *B. hordeaceus* to calculate Relative Interaction Indices (Armas *et al.*, 2004):

$$RII = (B_w - B_0) / (B_w + B_0),$$

in which B_0 corresponds to the biomass of a control individual, and B_w corresponds to the biomass of a full sibling individual grown in competition. RII has defined limits (-1, +1), with more negative values indicating stronger competition, and positive values indicating the strength of mutualistic interactions. We used RII's to test for differences among seed ancestries for each species via General Linear Models in R 3.1.2 (R Development Core Team 2010), with RII as the variable and each of the four possible combinations of *C. solstitialis* seed ancestry (pollen donor and pollen receiver) as the fixed treatment factor. Separate tests were used for *C. solstitialis* or *B. hordeaceus* biomass derived data. Tukey post-hoc tests were run when required by using the *glht* procedure in R's *multcomp* library.

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