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Competitive interactions are mediated in a sex-specific manner by arbuscular mycorrhiza in *Antennaria dioica*

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Running head: Competition in a dioecious species

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ABSTRACT

- Plants usually interact with other plants, and the outcome of such interaction ranges from facilitation to competition depending on the identity of the plants, including their sexual expression. Arbuscular mycorrhizal (AM) fungi have been shown to modify competitive interactions in plants. However, few studies have evaluated how AM fungi influence plant intraspecific and interspecific interactions in dioecious species.

- The competitive abilities of female and male plants of *Antennaria dioica* were examined in a greenhouse experiment. Females and males were grown in the following competitive settings: (i) without competition, (ii) with intrasexual competition, (iii) with intersexual competition, and (iv) with interspecific competition by *Hieracium pilosella* - a plant with similar characteristics to *A. dioica.* Half of the pots were grown with *Claroideoglomus claroideum,* an AM fungus isolated from the same habitat as the plant material. We evaluated plant survival, growth, flowering phenology, and production of AM fungal structures.

- Plant survival was unaffected by competition or AM fungi. Competition and the presence of AM fungi reduced plant biomass. However, the sexes responded differently to the interaction between fungal and competition treatments. Both intra- and interspecific competition results were sex-specific, and in general, female performance was reduced by AM colonization. Plant competition or sex did not affect the intraradical structures, extraradical hyphae, or spore production of the AM fungus.

- These findings suggest that plant sexual differences affect fundamental processes such as competitive ability and symbiotic relationships with AM fungi.

INTRODUCTION

Plant interactions are fundamental in shaping plant populations and communities (Schulze *et al*. 2005). Plants engage with other plants in complex ways that include both positive (i.e. facilitation) and negative (i.e. competition) interactions. Facilitation occurs when plants obtain physical protection from herbivores, higher attraction to pollinators or enhanced nutrient availability due to neighboring plants (Brooker *et al*. 2008; Callaway *et al*. 2002; Pugnaire *et al*. 2011). Competition occurs when neighboring plants decrease the survival, growth or fecundity of a given individual by reducing its access to light, nutrients or pollinators (Bazzaz 1996; Lambers *et al*. 2008).

Above- and belowground biotic interactions formed between the plants with other organisms can modify plant-plant interactions. One of the most important elements in the majority of terrestrial ecosystems is the mutualistic association between plants and arbuscular mycorrhizal (AM) fungi (Brundrett 2009). AM fungi improve plant growth through the increased uptake of nutrients (e.g. phosphorus, nitrogen) and water, and may also alleviate plant stresses caused by abiotic and biotic factors (Gehring & Bennett 2009; Miransari 2010). Therefore, AM have the potential to affect plant-plant interactions in two ways: first, through the connection of plant individuals into a common hyphal network, which may drain carbon or mineral nutrients from some individuals and support others (Hart *et al*. 2003). Second, if the sexes in dioecious plant species differ with respect to their response to AM fungi, their interaction with individuals of same sex, different sex or other species could be sex-specific.

The majority of plant species are hermaphroditic, but the complete separation of the male (i.e. pollen production) and female (i.e. seed production) sexual functions into separate individuals

has arisen several times in various plant lineages during plant evolution (Barrett 2002). In dioecious species, female and male individuals usually possess different resource demands associated with each sexual function and females typically invest proportionally more into reproduction than males (Delph 1999; Geber *et al*. 1999; Obeso 2002). This mode of resource allocation may lead to differences between the two sexes in terms of morphology, physiology, life-history traits (Delph 1999; Geber *et al*. 1999), and intensity of biotic interactions (Sánchez-Vilas *et al*. 2011), including the interaction with AM fungi (Vega-Frutis *et al*. 2013a). The plant-AM fungal relationship has been shown to be sex-specific. Females tend to have higher levels of AM colonization and benefit more from AM fungi in terms of growth and reproduction than males (reviewed in Varga 2010; Vega-Frutis *et al*. 2013a, b). However, the net outcome seems to be modified by abiotic factors such as drought (Varga & Kytöviita 2008), pH (Varga & Kytöviita 2010), and temperature (Vega-Frutis *et al*. 2014).

Few studies have evaluated sexual dimorphism in competitive abilities between the sexes of dioecious species (Table 1), and most of them only investigated intraspecific interactions (but see Graff *et al*. 2013; and Sanchez-Vilas *et al*. 2001). From this limited available evidence it is clear that dioecious plants are usually sexually dimorphic in terms of both intra- and interspecific competitive abilities. This difference has been linked to how the sexes allocate resources, especially during reproduction (e.g. Varga & Kytöviita 2012). Whether the sexes experience intrasexual competition or facilitation seems to depend both on the plant species and the parameter investigated (Table 1).

Even though most land plant species grow with AM fungi in their roots, the AM fungal influence on the outcome of competitive interactions in sexually dimorphic plants has largely been overlooked. Theoretically, AM fungi could affect competitive interactions in a sex-specific manner if one sex is able to obtain higher benefits from AM fungi than the other. In the present study, we examined the competitive ability of a dioecious species against a co-occurring hermaphrodite of similar growth form. The focal dioecious species, *Antennaria dioica,* is a low-growing species and is poorly adapted to compete with tall neighbours (pers. obs.). *Hieracium pilosella,* the competing

hermaphrodite in our experiment, directly competes for soil resources and indirectly by producing allelochemicals (Díaz-Barradas *et al*. 2015; Knicker *et al*. 2000). *Hieracium pilosella* is increasing in abundance in disturbed habitats such as roadsides in Northern Europe (Kiviniemi & Eriksson 1999), and is an invasive species in New Zealand and North America (Díaz-Barradas *et al*. 2015; Knicker *et al*. 2000). On the other hand, A. *dioica* is a red-listed declining species in Europe (Kalliovirta *et al*. 2010). Therefore, the interactions between these two species are of particular interest. Furthermore, we investigated whether AM fungi modify the response of the sexes of *A. dioica* growing in intra- and interspecific competitive settings. Specifically, we compared the growth of females and males growing alone, growing with other individual of the same sex (intrasexual interaction), growing with an individual of different sex (intersexual interaction) or growing with *H. pilosella* (interspecific interaction) either in symbiosis with AM fungi or without. There was little evidence for sexual dimorphism in intraspecific competitive ability in a previous experiment with *A. dioica* in the field (Varga & Kytöviita 2012). However, the two sexes have sex-specific responses to AM fungi (Varga & Kytöviita 2008; 2010). Taken these two previous results into account, we formulated two specific novel research questions: 1) is the competitive ability of female and male *Antennaria dioica* plants sex-specific*?* and 2) does AM symbiosis affect *Antennaria dioica* competitive interactions?

MATERIALS AND METHODS

Study species

Antennaria dioica (L) Gaertn (Asteraceae) is a dioecious, perennial herb that grows in nutrient-poor habitats such as heaths, dry grassland, sandy or stony places and forest margins. It is widely distributed in temperate to Arctic regions of the northern hemisphere (Tutin *et al*. 1976). It reproduces by seeds, and by clonal growth. Each individual plant (genet) can produce one to several

propagules (ramets) by clonal growth of surface crawling stolons, and generally each ramet may produce up to one flowering shoot. Female and male plants exhibit secondary sexual dimorphism: males produce more flowering shoots and inflorescences which are also heavier than the female ones even though there is variation among years and populations (Varga & Kytöviita 2011). In Finland, flowering occurs between June and July and the frequency of reproduction is similar between sexes (Varga & Kytöviita 2011). *Antennaria dioca* is pollinated by generalist insects (Willis & Burkill, 1903) and it produces small seeds that are easily dispersed by wind (Eriksson 1997). In addition, both sexes have been reported as mycotrophic in the field (Varga & Kytöviita 2011, 2012; Vega-Frutis *et al*. 2013b). The population sex ratio is usually female-biased, and there is no spatial segregation of the sexes (Öster & Eriksson 2007; Varga & Kytöviita 2011).

Hieracium pilosella L. (Asteraceae) is a hermaphroditic, stoloniferous perennial herb that produces distinct rosettes on a slender rootstock, it produces sexual and asexual seeds that are dispersed by wind (Krahulcova & Krahulec 1999), and glandular hairs covering all vegetative organs (Bishop & Davy 1994). *Hieracium pilosella* is Native to Europe and Eastern Asia, but is an invasive species in New Zealand and North America (Díaz-Barradas *et al*. 2015; Knicker *et al*. 2000). This species grows well on sunny areas such as sandy soils and similar nutrient poor habitats. We selected this species because it partially occupies the same habitats as *A. dioica* in Finland (pers. obs.). Moreover, both species have similar life history and flowering phenology, and are usually colonized by AM fungi in the field (Díaz-Barradas *et al*. 2015; Read *et al*. 1976; Varga & Kytöviita 2011; Vega-Frutis *et al*. 2013b).

Experimental setup

In August 2011, we randomly collected 24 female (hereafter A♀) and 28 male (hereafter A♂) *A. dioica* genets, and 20 *H. pilosella* (hereafter Hp) genets from the same location in central Finland (62° 3' 10" N, 25° 32' 48" E). We divided each plant into several clonal fragments (ramets) and propagated them in individual pots filled with ~250 g of sterilized sand in greenhouse with a light period of 18 h light/6 h darkness, and 26 °C light/20 °C dark temperature conditions at the University of Jyväskylä. To ensure that the plants were not colonized by field AM fungi, we removed all root systems before potting the ramets. The ramets were allowed to grow and develop new root systems for four months. A commercial liquid fertilizer (Substral® vita+plus, 7 mL per liter given following manufacturer's instructions, containing N:P:K 6:1.3:5 and micronutrients Cu, Fe, Mn, MO, and Zn) was given in three occasions to stimulate growth, and the seedlings were watered with tap water about three times per week.

The experiment was started in December 2011. Each experimental pot contained either one female or one male *A. dioica* ramet (the "sex" of the focal species), and was allocated to the following competition settings (Fig. S1): no competition (A♀or A♂growing alone, 44 pots); Intrasexual competition (A♀and A♂growing with another *A. dioica* plant of the same sex, A♀A♀and A♂A♂, 44 pots); Intersexual competition (A♀and A♂growing with another *A. dioica* plant of the opposite sex, A♀A♂and A♂A♀, 22 pots); Interspecific competition (A♀and A♂growing with *H. pilosella*, A♀Hp and A♂Hp, 44 pots). Therefore, the experiment consisted of 264 plants (110 A♀, 110 A♂, and 44 Hp) distributed in 154 pots.

The experimental plants were weighted and the number of ramets were counted before potting. We selected plants of similar size to ensure we did not get effects due to differences in the initial plant biomass. Consequently, in the beginning of the experiment females and males of *A. dioica*, and *H. pilosella* did not differ in fresh biomass or the number of ramets (see data analyses and results). The pots were filled with 1053 cm^3 of a soil mixture containing autoclaved soil, sand

and perlite (1:1:1). The soil was originally collected from the same location where the plants were collected and had a pH value of 7.4 (ISO 10390; 2005), 1.4% organic matter (SFS 3008), <0.1% total nitrogen (Kjeldahl test), 0.05% total phosphorus (HNO_3 dissolution + ICP-OES), and 0.1% total potassium (HNO_3 dissolution + ICP-OES). Soil chemical analyses were carried out by the Institute for Environmental Research (Jyväskylä, Finland). To improve soil fertility, we added 1.5 g L^{-1} of bone meal (Äetsä Trading Co, Äetsä, Finland) to the mixture.

We allocated half of the pots to the mycorrhizal treatment (referred to as AM plants). Each pot was inoculated with about 100 spores of *Claroideoglomus claroideum* suspended in 1 mL of water (Varga & Kytöviita 2008, 2010). The other half of the pots was allocated to the non-mycorrhizal treatment (referred to as NM plants) and received 1 mL of inoculum-wash without AM spores. All the pots received an additional 1 mL of a soil microbial suspension filtered through a 5.0 µm nitrocellulose Millipore filter to partially return the microflora to the sterilized soil. The microbial suspension was obtained from the inoculum-washing water when preparing the AM inoculum. The experimental plants were grown in greenhouse (light period of 18 h light/6 h darkness, and 26 °C light/20 °C dark temperature) at the University of Jyväskylä, and we rotated the pots every two weeks. The plants were watered with tap water when needed (about three times per week to reach field capacity).

The *Claroideoglomus claroideum* isolate was originally isolated from Ylistaro in central Finland (62° 57’ N, 22° 31’ E). The AM inoculum was propagated prior the experiment in pots filled with sterilized sand under greenhouse conditions (light period of 18 h light/6 h darkness, and 26 °C light/20 °C dark temperature) at the University of Jyväskylä. *Plantago lanceolata* was used as host plant, and after six months the spores were washed from the potting media, collected on a 50 µm sieve, and diluted in water to obtain the AM inoculum. This AM fungal species is common in Finland, and has been also isolated from sites where *A. dioica* is the predominant plant (Vega-Frutis *et al*. 2013c). Previous studies have shown that an isolate of *C. claroideum* provides benefits to the plant

species tested so far under experimental conditions (Kytöviita *et al*. 2003, Kytöviita & Ruotsalainen 2007), and the studies with *A. dioica* have shown that the sexes gain a different benefit from the same mycorrhizal symbiont (Varga & Kytöviita 2008, 2010).

Plant parameters

During the experiment, we recorded plant survival and flowering. The plants were harvested after four months (April 2012). At that point, we scored the number of ramets, and measured the average spacer length (i.e. the distance in mm between the initial ramet and all adjacent produced ramets) in *A. dioica.* Subsequently, the aboveground biomass (ramets and floral shoot biomass), and belowground biomass (roots) were separated and oven-dried in paper bags at 60°C for 3 days, and then the root/shoot ratio was calculated as belowground/aboveground dry biomass. We also took a root sample to assess mycorrhizal colonization before drying the roots (see below).

Mycorrhizal plant benefit, defined as the performance ratio between mycorrhizal and non-mycorrhizal plants (Kytöviita *et al*. 2003), was calculated for the total biomass, belowground biomass, aboveground biomass, root/shoot ratio, number of ramets and spacer length. Given that we did not have the same genotypes represented in all treatments, and because there were no differences at the beginning of the experiment between female and male *A. dioica*, and *H. pilosella* plants in total fresh plant biomass and number of ramets, we paired AM and NM plants randomly to calculate the mycorrhizal plant benefit within the competition treatment. Ratios >1 indicate that AM fungal symbiosis was beneficial, <1 indicate that the symbiosis was detrimental, and ratios close to one indicate no net mycorrhizal benefit for the plants.

Fungal parameters

We measured three fungal parameters: the colonization of intraradical AM fungal structures (hyphae, vesicles and arbuscules), the length of extraradical hyphae (EH), and the final number of spores. To estimate the production of intraradical AM fungal structures, the roots were processed according to the method of Koske & Gemma (1989), and stained with trypan blue (0.05%). We estimated the colonization by AM fungi based on 30 root fragments (~15 mm long) from each plant. Each root fragment was examined at three equally spaced points under a light microscope at 100x and 400x total magnification, using the cross-hair intersection method (McGonigle *et al*. 1990). The presence/absence of fungal structures was used to calculate the percentage of root colonized by AM fungi: positive counts were summed and divided by the total number of points observed.

To estimate the production of EH, soil from the center of each pot was collected and oven-dried (60°C for 3 days). Length of EH was measured by the filtration-gridline method (Sylvia 1992). A sample of 5 g of dry soil was suspended in a mixture of 100 mL of de-ionized water and 15 mL of sodium hexametaphosphate (5%) for 10 minutes to disperse soil particles, and subsequently shaken vigorously using a blender for 30 seconds to homogenize the soil suspension. The sample was poured on a 45 μm sieve and washed with abundant water. The material recovered from the sieve was transferred to a beaker, mixed with 200 mL of water and allowed to settle for 1 minute. A 10 mL aliquot was transferred on a 0.8 μm membrane filter (Schleicher & Schuell) and vacuum-filtered. After filtering off the water, the hyphae were stained with 4 drops of trypan blue (0.05%) for 5 minutes and then vacuum-filtered again. The membrane was transferred to a microscope slide and mounted with polyvinyl alcohol–lacto–glycerol solution. The length of EH was estimated using a grid in the eyepiece of light microscope at 312.5x total magnification. Extraradical hyphae were identified as aseptate hyphae and stained blue. Each membrane was examined in 10 random points, and the number of hyphae crossing the grid lines incorporated in the microscope were applied into Newman's formula (Newman 1966): $LEH = (\pi NA/2H) \times D$, where *LEH* is the length of EH in

centimeters, *N* is the number of intersections, *A* is the active area of membrane filter (1.77 cm^2), *H* is the total length of grids (10 grids = 8 cm) and *D* is the dilution factor (200 mL).

AM fungal spores were extracted from 50 g of dry soil samples per pot by wet sieving and decanting (Gerdemann & Nicolson 1963) using 500 µm and a 45 µm sieves, followed by two centrifugation cycles: first with water at 3000 rpm for 5 minutes, and after that the sediment was resuspended in 70% sucrose solution and centrifuged at 2500 rpm for 2 minutes. All spores in the 50 g sample of soil were counted at 12x magnification with a stereomicroscope.

Data analyses

All statistical analyses were performed with the statistical software R (R Development Core Team 2015). First we performed a graphical data exploration (Zuur 2010) to assess the potential best models with which to analyze each type of variable. For all models, we reviewed that the residuals were normally distributed, the variances homogeneous, and when necessary data transformations were used (see below).

To test for differences in initial fresh biomass between sexes of *A. dioica* and *H. pilosella*, we used one-way analysis of variance (ANOVA). To meet the model assumptions the initial fresh biomass was square root transformed. Differences in the initial number of ramets among plants were analyzed with a generalized linear model (GLM) fitted with negative binomial error structure, and log- as link function (library 'MASS').

The effect of sex (F, M), fungus (AM, NM), competition (alone, intrasexual, intersexual, interspecific), and the interaction among these three factors on belowground biomass, aboveground biomass, total plant biomass, root/shoot ratio, and ramets spacer length were tested with three-way ANOVAs. Differences in plant survival, proportion of flowering plants and the number of ramets produced were tested with GLMs. Models on plant survival and proportion of flowering plants were

fitted with binomial error structure and logit link function, and for the number of ramets, a Poisson error structure and log- link were used. To meet the model assumptions, the below-, aboveground, total plant biomass and root/shoot ratio were square root transformed. Significant differences between the levels of a factor (i.e. competition) or two-way interactions were tested with Tukey's test, and *a posteriori* contrasts based on *t*-test (library 'lsmeans') for ANOVA and GLM respectively. When the triple interaction was significant, we tested the effect of fungus, competition and the interaction between these two factors separately for female and male plants.

We used ANOVAs to explore differences in mycorrhizal plant benefit. Plant sex, competition, and the interaction between these factors on belowground biomass, aboveground biomass, total plant biomass, root/shoot ratio, number of ramets and spacer length were tested. To meet the model assumptions a logarithmic transformation was applied to the variables. Significant differences between the levels of the factor competition (alone, intrasexual, intersexual, interspecific) were tested with Tukey's test.

ANOVA and GLM were used to explore differences in the fungal parameters. The effect of sex, competition, and the interaction between these two factors on the colonization percentage by hyphae was tested with two-way ANOVA. To meet the model assumptions, the percentage of hyphae was arcsine transformed. Vesicles and arbuscules were analyzed as binary variables (presence/absence) because of their low frequencies in the roots. For the binary variables, we used GLMs fitted with a binomial error structure and logit link. The effect of competition and sex on the extraradical hyphae per pot was tested with one-way ANOVA, while a GLM fitted with negative binomial, and log link was used to test the effect of competition and sex on the number of spores per pot.

RESULTS

Plant parameters

Initial biomass and number of ramets

We selected plants of similar size to ensure we did not get effects due to differences in the initial plant biomass. In the beginning of the experiment females and males of *A. dioica* and *H. pilosella* did not differ in their total plant biomasses (A♀0.77 ± 0.04 g, A♂0.77 ± 0.03 g, and Hp 0.72 ± 0.06 g; $F_{2,261} = 0.664$, $P = 0.520$) or in the number of ramets (A♀1.3 ± 0.1, A♂1.3 ± 0.1, and Hp 1.2 ± 0.1; $\chi^2_{2,261} = 0.420$, $P = 0.810$).

Survival

During the experiment, 15 males and 9 females of the 220 *A. dioica* plants died, and there were no statistically significant differences between sexes ($\chi^2_{1,176} = 1.909$, $P = 0.167$), fungal treatment ($\chi^2_{1,175} = 0.869$, $P = 0.351$), competition treatment ($\chi^2_{3,172} = 0.532$, $P = 0.911$) or their interactions (all $P \geq 0.270$) explaining the mortality. The dead plants were excluded from further analyses. In addition, eight plants (two A♀and six A♂) were also excluded because their neighbors died during the experiment. Finally, 32 more plants (14 A♀and 18 A♂) from the AM treatment were excluded because at the end of the experiment they did not show any sign of AM colonization in their roots. Therefore, the final sample size was 156 plants (85 A♀and 71 A♂) in 112 pots.

Plant growth

Over the 120 experimental days, competing plants accumulated 37% less total biomass than plants growing alone regardless of the particular type of competition (competition main effect, $F_{3,136} = 11.260$, $P < 0.01$). Moreover, mycorrhizal plants accumulated 20% less total biomass than NM plants

(fungus main effect, $F_{1,136}$ = 8.295, P = 0.004). However, the sexes responded differently to the fungal and competition treatments (Sex × Fungus × Competition interaction: $F_{3,136}$ = 3.878, P = 0.010). Specifically, when analyzed separately for each sex, only the competition treatment affected the total ($F_{3,65}$ = 8.255, P < 0.01), and aboveground ($F_{3,65}$ = 8.916, P < 0.01) biomass in males. In females, however, a significant biomass reduction due to AM fungi was detected when plants competed with other females or with *H. pilosella* (fungus x competition interaction: $F_{3,71}$ = 3.032, P = 0.03; Fig. 1A). The fungal treatment did not affect plant biomass in males ($F_{1,65}$ = 2.130, P = 0.149 and $F_{1,65}$ = 1.054, P = 0.374 for the main effect of fungal treatment and for the fungus x competition interaction; Fig. 1B). Similar patterns were observed when plant biomass was divided into above- and belowground biomasses (Table 2, Fig. S2 and S3). There were no significant differences between sexes and fungal treatments explaining the root/shoot ratio (Table 2). However, the competition treatment affected the root/shoot ratio (Table 2), and the plants growing with *H. pilosella* had significantly higher root/shoot ratio (0.139 ± 0.011) compared with the other competition treatments (no competition: 0.102 ± 0.004, intrasexual: 0.105 ± 0.003, intersexual: 0.101 ± 0.004).

Number of ramets and spacer length

Even though the sexes did not differ in the number of ramets at the end of the experiment (A♀5.7 ± 0.3 *vs* A♂5.3 ± 0.3; GLM: deviance$_{1,150}$ = 0.922, P = 0.337), the plants growing without competitors produced significantly more ramets (deviance$_{3,146}$ = 58.20, P < 0.001, Fig. 2A), and the interaction between mycorrhiza and competition affected ramet production (deviance$_{3,139}$ = 11.379, P = 0.009). Particularly, the number of ramets was reduced in mycorrhizal plants when growing in intrasexual competition (Fig. 2A).

Spacer length was on average 0.8 mm greater in females than males (A♀65.5 ± 2.3 mm *vs* A♂ 57.5 ± 1.6 mm; $F_{1,99}$ = 8.522, P < 0.01) and competition affected spacer length significantly ($F_{3,99}$ = 3.264, P = 0.024; Fig. 2B). Plants growing alone had the greatest spacer length and plants growing in interspecific interaction the shortest (Fig. 2B). AM fungi did not affect the spacer length significantly ($F_{1,99}$ = 0.890, P = 0.765).

Flowering

During the course of the experiment 38% of plants flowered. A significantly larger proportion of males flowered compared to females (♂54.8% ± 5.9 *vs* ♀22.8% ± 4.8, GLM: deviance$_{1,150}$ = 16.780, P < 0.001) irrespectively whether the plants were growing alone or in competition (deviance$_{3,146}$ = 1.300, P = 0.727). AM symbiosis decreased the proportion of flowering plants (from 44.0% ± 5.2 to 29.5% ± 5.9, deviance$_{1,149}$ = 4.00, P = 0.045, Fig. S4). All interactions among factors were statistically non-significant (Table S1).

Mycorrhizal plant benefit

The sexes differed in the benefit obtained by AM inoculation in terms of growth, but only in interspecific competition (Fungus × Competition interaction, Figs. 3A-C, E). For female plants competing with *H. pilosella*, mycorrhizal benefit in biomass accumulation was lower than 1, but the mycorrhizal benefit was close or > 1 in males. This indicates that female *A. dioica* grown with *H. pilosella* suffered from being colonized by AM, but in males no significant growth increase or decrease was observed under interspecific competition. The females growing without competitors tended to have higher mycorrhizal plant benefit than males, although no statistically significant differences were detected (Figs. 3A-C, E). Competition treatments affected mycorrhizal benefit in terms of number of ramets produced (Table 3, Fig. 3E). Overall, the mycorrhizal benefit in this

parameter was higher when plants were grown alone when compared with plants in intra- and intersexual competition. The AM benefit in the root/shoot ratio or spacer length was close to 1 and we did not find significant differences in any factor analyzed (Table 3, Figs. 3D and F).

Fungal parameters

Intraradical fungal colonization

A similar proportion of root length was colonized by AM hyphae regardless of the sex of the plants (A♀13.4% ± 1.6 and A♂18.5% ± 2.4; $F_{1,50}$ = 2.872, P = 0.096) or the competition treatment (17.4% ± 3.0, 15.2% ± 2.4, 16.8% ± 4.7 and 14.3% ± 1.6 for *A. dioica* plants grown alone, with intrasexual, intersexual and interspecific competition respectively; $F_{3,50}$ = 0.241, P = 0.867), and there was no interaction between these two factors ($F_{3,50}$ = 0.659, P = 0.580).

The presence of vesicles was observed in 58% of all mycorrhizal *A. dioica* plants regardless of their sex or competition treatment (Table S2). The presence of vesicles was not affected by plant sex, competitive setting or their interaction (Table S2). Arbuscules were observed in 44% of mycorrhizal *A. dioica* plants, and again no differences due to plant sex, competition or their interaction were detected (Table S2).

Extraradical hyphae and spore production

At the end of the experiment, both the amount of EH ($F_{3,45}$ = 0.426, P = 0.735) and the number of spores present per pot (GLM: deviance $_{3,45}$ = 0.803, P = 0.845) were similar among pots and not related to the competition treatment (Table S3).

DISCUSSION

Relative intensity of intra- and interspecific competition is important in explaining species coexistence (Silvertown 2004). In the present study, *Hieracium pilosella* strongly reduced *Antennaria dioica* growth, and interspecific competition for belowground resources was stronger than intraspecific competition. This is in line with field studies where *H. pilosella* generally wins over *A. dioica* (Eriksson 1997). The superior competitive ability of *H. pilosella* has been linked with its capacity to control and sequester N supply, through modifying soil microbial C and N concentrations, and affecting the soil organic matter cycle (Saggar *et al*. 1999). This, possibly along with allelopathic compounds released to the soil results in a "halo" around *H. pilosella* plants that seems inhabitable to other plant species (Makepeace *et al*. 1985, Saggar *et al*. 1999).

Lovett-Doust (1981) described two competition strategies where species are morphologically adapted to competition intensity. 'Phalanx'-plants have short ramet distances and are adapted to high competition environments. 'Guerilla'-plants have long distances between ramets and this strategy should be more advantageous when competition intensity is low (Lovett-Doust 1981). Although originally developed to describe interspecific competitive scenarios, these strategic responses can also be applied to evaluate plastic responses within species. In the present work, the spacer length was reduced in *A. dioica* when competing with *H. pilosella* suggesting plastic 'phalanx' response to interspecific competition. Female *A. dioica* had longer spacers than males as previously observed by Vega-Frutis *et al*. (2014) suggesting that females may compete for new space more aggressively than males. Alternatively, female *A. dioica* plants could theoretically occupy microhabitats where competition intensity is lower than those occupied by males. However, the latter scenario is not supported by a field study that shows that the distribution of the sexes is not spatially segregated (Varga & Kytöviita 2011).

The coexistence of the two sexes in dioecious species is a special case of intraspecific interaction that cannot be simply extrapolated from models explaining coexistence of

hermaphrodite individuals. This is because resource acquisition and allocation patterns may differ between the sexes in dioecious species. Plant roots respond to interspecific and intraspecific competition differently, and actively avoid root elongation to the root space occupied by the same species (Gruntman & Novoplansky 2004). Within species, plants can recognize the identity of neighboring individuals (Fang *et al*. 2013), and a dioecious plant has been observed to detect the sex of the competitors (Mercer & Eppley 2014). Even though untested, it seems possible that dioecious plants can also recognize the sex of intraspecific roots. Due to the different costs of seed and pollen production, resource demand differences in sexual reproduction in dioecious species have been observed (Delph 1999). Therefore, reproductive synergy and niche differences between sexes could select for intersexual competition to be less severe than intrasexual. In contrast to this, intersexual competition is previously reported either equal to intrasexual or more intense than intrasexual (Table 1). In the present study, a larger proportion of male plants flowered during the experiment in comparison to females as previously observed under greenhouse conditions (Varga & Kytöviita 2012, Vega-Frutis *et al*. 2014). Therefore, it is likely that during the first year the cost of reproduction in females is higher than in males, presumably to support the reproduction by seed. However, Varga & Kytöviita (2011) monitored the flowering frequency for five consecutive years in the field and they found that the accumulated flowering frequency was similar between sexes.

Females and males had similar mortality rate. As far as we know, only two other studies have directly measured plant survival in response to the sex of the competitor (Table 1). In line with the present study, no sex-specific differences in plant survival in response to the sex of the competitor were observed in *A. dioica* or *Distichlis spicata* (Table 1). However, sex-specific adult *A. dioica* mortality rates have been reported previously in response to environmental factors and mycorrhiza (Varga & Kytöviita 2010). It remains to be tested how environmental factors and mycorrhiza affect mortality when plants are also facing competition - a prevailing situation in nature.

Recently, the role of symbiosis in plant competition and coexistence patterns has been acknowledged (Lin *et al*. 2015). When a mycotrophic species is competing against a non-mycotrophic one, AM fungi shift the competitive balance in favor of the mycotrophic species (Lin *et al.* 2015). However, when two mycotrophic species compete with each other, as in the present study, the outcome is more difficult to predict and depends on the identity of plant and AM fungal species, and environmental conditions (Lin *et al*. 2015). The two target species here are highly mycotrophic and AM fungi have been previously shown to improve the competitive ability of *Hieracium pilosella* (Höpfner *et al*. 2015). In the present case, mycorrhizal benefit was reduced in female but not in male *A. dioica* when competing against *H. pilosella*. This suggests that the female and male *A. dioica* plants differ in their interspecific competitive ability under field conditions where plants are mycorrhizal as a rule. Female plants generally have higher mycorrhizal frequency in their roots as well as gain higher benefits from AM symbiosis compared to males (Varga 2010; Vega-Frutis *et al*. 2013a). Furthermore, the sex ratio of *A. dioica* is heavily female biased under field conditions (Varga & Kytöviita 2011). Therefore, the reduction of female but not male *A. dioica* growth performance by mycorrhizal symbiosis against *H. pilosella* is unexpected. Likely, female plants allocated more resources to growth than their symbiont under interspecific competition, therefore, facilitating the growth and mycorrhizal colonization in *H. pilosella* (negative feedback, Johnson *et al*. 2006). Another explanation includes differences in root morphological traits such as first-order root diameter, specific root length and root branching ratio. All these root traits are important determinants of mycorrhizal colonization (Kong *et al*. 2014), and although there are no studies evaluating the root traits in our study species, it is likely that these root traits differ between species, and these traits affect the observed host-AM preference (Helgason & Fitter 2009). Therefore, AM fungal identity and host plant can affect plant mycorrhizal benefit in competitive settings. The *Claroideoglomus claroideum* isolate used in the present experiment has benefitted female *A. dioica* individuals in previous experiments (Varga & Kytöviita 2008, 2010), and did so also in the present experiment

when the plants were not competing. The unexpected sexual difference in mycorrhizal benefit when exposed to heterospecific competition highlights the complexity of biotic interactions.

Even though the symbiosis with AM fungi is generally considered beneficial to plants, in reality the interaction between plants and AM fungi ranges from detrimental to beneficial depending on the plant-fungal combination and the abiotic conditions experienced by the symbionts (Johnson & Graham 2013). Keeping in mind the capacity that *H. pilosella* has to modify mineral soil nutrients, the absence of mycorrhizal plant benefit under interspecific competition, in the present study, could be due to changes in the relative competition for soil nutrients between species. Similar results have been previously reported (e.g., Li *et al*. 2008, see Smith & Smith 2011, 2012).

Theoretically, the symbiotic fungus could benefit of accessing more diverse and more numerous host plants as these could provide more carbon to the fungus than single plants (van der Heijden *et al*. 1998). In the present experiment, the mycorrhizal fungus did not appear to benefit of having more numerous or more diverse host plants in the pot, and the amount of extraradical hyphae and the spore production were not affected by competition. Although the frequency of intraradical fungal structures was higher in symbiosis with *H. pilosella,* this did not translate into higher extraradical hyphae or more numerous spores. This suggests that the symbiosis with *H. pilosella* was not more beneficial to the fungus than that with *A. dioica*. *Hieracium pilosella* growing in the same pot with *A. dioica* females had almost twofold root colonization rate by hyphae (A♀: 11.8 ± 3.9%, and *Hp* 21.2 ± 7.5%), while when *A. dioica* males were growing with *H. pilosella* the difference in the hyphal colonization rate was minor (A♂: 14.1 ± 4.7%, and *Hp* 19.6 ± 6.5%). None of these differences in root colonization affected the spore production.

In conclusion, the interaction between the symbiotic fungus and the host plant is modified by neighbouring plants. The sex of the plant had profound effects on plant competitive ability. Female sex was sensitive to mycorrhizal symbiosis and competitor identity, while the male sex had better performance regardless of symbiosis and neighbor identity. Higher sensitivity of females to

environmental conditions such as drought and pH has been previously shown (Varga & Kytöviita 2008, 2010). Altogether, females show more plastic response to variations in both biotic and abiotic conditions. At optimal conditions, the sexes perform similarly, but when stressed female growth and survival is reduced. The reasons for higher plasticity in females could be related to the predicted higher cost of reproduction in females, and resource limitation of seed production under stressful conditions. This is supported by the lower flowering frequency in females in comparison to males in the present experiment, and when competing under field conditions (Varga & Kytöviita 2012). Under natural conditions, if females and males differ in their competitive ability, and the flowering frequency is decreased in females, this could result in a decrease of potential sexual reproduction, given that dioecious plant are obligately outcrossed. Therefore, dioecious plant may be more prone to extinction (Vamosi & Otto 2002, Vamosi & Vamosi 2005). However, the exact evolutionary and ecological mechanisms resulting in higher plasticity in response to competition by female *A. dioica* warrant further targeted experiments in dioecious species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

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Table 1: studies evaluating the intraspecific competitive abilities of the sexes in dioecious plants.

Species and family	Reference	Life form	SSS	Type of study	Plant parameter measured		Sex	Competition
Antennaria dioica (Asteraceae)	Varga and Kytöviita 2012	Herb	No	Common garden	Survival		ns	ns
					Growth	#Ramets	ns	NO > SA = DI
						Aboveground biomass	ns	NO > SA = DI
						Belowground biomass	ns	NO = DI > SA
						RGR	ns	NO = DI > SA
					Reproduction	Proportion of flowering plants	F: NC > SA, DI = SA = NO M: ns	
						#Inflorescences	M > F	NO > SA = DI
Antennaria dioica (Asteraceae)	This study	Herb	No	Greenhouse	Survival		ns	ns
					Growth	#Ramets	ns	NO > SA = DI*
						Aboveground biomass	ns	NO > SA = DI*
						Belowground biomass	ns	NO > SA = DI*

					Reproduction	Proportion of flowering plants	M > F	ns
Distichlis spicata (Poaceae)	Mercer and Eppley 2010	Grass	Yes	Field (reciprocal transplant)	Survival		ns	ns
					Growth	Plant biomass	ns	SA > DI = NO
						Root/Shoot	ns	DI ≥ SA ≥ NO
					Reproduction		-	-
Distichlis spicata (Poaceae)	Rogers and Eppley 2012	Grass	Yes	Greenhouse	Survival		-	-
					Growth	Plant biomass	ns	NO > SA = DI
						Root/Shoot	M > F	DI > NO = SA
					Reproduction		-	-
Distichlis spicata (Poaceae)	Mercer and Eppley 2014	Grass	Yes	Lab (liquid medium)	Survival		-	-
					Growth	Plant biomass	M < F	SA = NO > DI
						Root/Shoot	ns	DI > SA = NO
					Reproduction		-	-
Lindera melissifolia (Lauraceae)	Hawkins *et al*. 2009	Tree	?	Greenhouse	Survival		-	-
					Growth	Stem height, Leaf number	F: ns M: NO > SA	

					Reproduction		-	-
Mercurialis annua (Euphorbiaceae)	Hesse and Pannell 2011	Annual herb	?	Greenhouse, two nutrient levels	Survival		-	-
					Growth	Aboveground biomass	F > M	NO > SA = DI
					Reproduction	Reproductive allocation	M: ns F: SA ≥ DI ≥ NO	
Populus cathayana (Salicaceae)	Chen *et al*. 2014	Tree	Yes	Greenhouse, two watering regimens	Survival		-	-
					Growth	Total biomass	F: ns M: SA > DI	
					Reproduction		-	-

Notes: Only studies with a control for the competition treatment (i.e. plants growing without competition) are included. When the effect of Sex or Competition were significant, the direction of the effect is indicated. If the Sex × Competition interaction was significant, differences in competition treatment are indicated separately for each sex. *: Mycorrhizal effects detected.

DI: plant growing with the different sex (i.e. intersexual competition); NO: plants growing without competition; SA: plants growing with the same sex (i.e. intrasexual competition); SSS: Sexual Spatial Segregation; ns: non-significant differences reported.

RGR = Relative Growth Rate

Table 2: ANOVA results for plant growth parameters in *Antennaria dioica*. Significant values are shown in bold.

Source of variation		Belowground biomass (g)		Aboveground biomass (g)		Root/shoot ratio	
	dfs	*F*	*P*	*F*	*P*	*F*	*P*
Sex	1	3.079	0.081	1.489	0.224	1.767	0.186
Fungus (Fun)	1	6.830	**0.009**	8.421	**0.004**	0.158	0.690
Competition (Comp)	3	5.776	**<0.001**	11.972	**<0.001**	8.318	**<0.001**
Sex × Fun	1	2.895	0.091	1.303	0.255	0.079	0.779
Sex × Comp	3	0.141	0.934	0.077	0.971	1.208	0.309
Fun × Comp	3	0.393	0.758	0.823	0.482	1.063	0.366
Sex × Fun × Comp	3	2.470	0.064	4.020	**0.009**	0.887	0.449
Residuals	136						

Table 3: ANOVA results for mycorrhizal plant benefit in *Antennaria dioica*. Significant values are shown in bold.

Source of variation		Belowground biomass (g)		Aboveground biomass (g)		Total biomass (g)		Root/shoot ratio		Number of ramets		Spacer length (mm)	
	dfs	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Sex	1	2.746	0.103	1.045	0.311	1.148	0.289	1.150	0.288	0.150	0.699	2.813	0.099
Competition (Comp)	3	1.594	0.202	2.043	0.119	1.942	0.135	0.489	0.691	4.934	**0.004**	0.217	0.884
Sex x Comp	3	3.768	**0.016**	4.813	**0.005**	4.852	**0.004**	1.657	0.188	2.674	0.057	0.816	0.490
Residuals	49