Competitive ability of a tetraploid selfing species (*Capsella bursa-pastoris*) across its expansion range and comparison with its sister species

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Abstract

Self-fertilization is often associated with ecological traits corresponding to the ruderal strategy in Grime's CSR classification. Consequently, selfers are expected to be less competitive than outcrossers, either because of a colonization/competition trade-off or because of the deleterious genetic effects of selfing. Range expansion could reduce further competitive ability while polyploidy could mitigate the effects of selfing. Although suggested by meta-analyses, these predictions have not been directly tested yet. We compared the competitive ability of four *Capsella* species differing by their mating system and ploidy level. We found that the two diploid selfing species (*C. rubella* and *C. orientalis*) were more sensitive to competition than the diploid outcrosser (*C. grandiflora*), and the tetraploid selfer (*C. bursa-pastoris*) was intermediate. Within *C. bursa-pastoris*, we also found that sensitivity to competition increased across range expansion. These results highlight the possible roles of ecological context and ploidy in the evolutionary trajectories of selfing species.

Introduction

The transition from outcrossing to selfing is very common in flowering plants. It is likely that many shifts to selfing abort early because of the rapid deleterious effect of selfing (Willi 2013; Griffin & Willi 2014; Abu Awad & Billiard 2017). But, if successful, the evolution of selfing is often associated with habitat shift and range expansion, thanks to the reproductive assurance offered by the ability to self (Randle *et al.* 2009; Grossenbacher *et al.* 2015). Selfers are more frequently found under conditions in which obligatory outcrossers pay the demographic cost of mate limitation, such as disturbed and patchy habitats or newly available environments (Baker 1967; Munoz *et al.* 2016). In addition to reproductive traits, often referred to as the "selfing syndrome" (Sicard & Lenhard 2011), other traits may thus evolve after the transition to selfing in relation with new ecological conditions. For instance, selfing is often associated with weedy habit (Clements *et al.* 2004) and invasiveness (van Kleunen *et al.* 2008). More generally, when referring to Grime's CSR ecological strategies, an excess of selfers is observed among ruderal species, corresponding to early colonizers in ecological successions, whereas more competitive species tend to be outcrossers (Munoz *et al.* 2016).

At least two mechanisms could explain the negative association between selfing and competitiveness. First, a colonization/competition trade-off could be involved, where selection for better colonizing ability would be at the cost of lower competitive ability (Burton *et al.* 2010). Related to this hypothesis, if costly, traits involved in competitive ability could be selected against during range expansion when low competitive environments are colonized (Bossdorf *et al.* 2004). Alternatively, selfing

could affect competitive ability through its negative genetic effects, especially the accumulation of weakly deleterious mutations. Deleterious mutations affecting the efficiency of resource acquisition are predicted to reduce competitive ability (Agrawal 2010; Agrawal & Whitlock 2012). Deleterious mutations can accumulate in selfers because of linked selection effects (Wright *et al.* 2008; Glémin & Galtier 2012) but also because of recurrent bottlenecks followed by demographic expansion, the so-called "expansion load" (Peischl *et al.* 2013; Peischl & Excoffier 2015). In agreement with a possible effect of deleterious mutations on competitive ability, inbred individuals have been found to suffer more from competition than outbred ones (Cheptou *et al.* 2000; Cheptou *et al.* 2001; Yun & Agrawal 2014). Polyploidy is another factor that can alter the above predictions. Polyploidy is often associated with selfing/self-compatibility (Barringer 2007; Robertson *et al.* 2011) and it is supposed to increase competitiveness (Comai 2005; te Beest *et al.* 2012) and to reduce the genetic load, at least for a transient period after the formation of the polyploid species (Otto & Whitton 2000). Thus, polyploidy could buffer the deleterious effects of selfing. According to the rationale presented above, and whatever the underlying causes, we predict that (i) transition from outcrossing to selfing should lead to reduction in competitive ability, (ii) this effect should be less pronounced in polyploid than in diploid selfers, and (iii) within species, competitive ability should decline during range expansion.

The *Capsella* genus (Brassicaceae) is a good model to address these questions. It comprises four closely related species with contrasting mating systems and ploidy levels. The only outcrossing species in the genus, *C. grandiflora* (Fauché & Chaub.) Boiss. is restricted to western Greece and Albania but has a large effective population size, with strong evidence of efficient positive and purifying selection (Foxe *et al.* 2009;

Slotte *et al.* 2010; Williamson *et al.* 2014). The three selfing species include two diploids: *C. orientalis* Klokov diverged from *C. grandiflora* about one million years ago and *C. rubella* Reut. much more recently (30,000 – 50,000 years ago Foxe et al., 2009, Guo et al., 2009). They have much larger species range but much lower effective population size and have accumulated weakly deleterious mutations (Foxe *et al.* 2009; Guo *et al.* 2009; Slotte *et al.* 2013; Douglas *et al.* 2015; Kryvokhyzha *et al.* In prep). The third selfing species, *C. bursa-pastoris* (L.) Medik., is an allo-tetraploid with disomic inheritance that originated about 200,000 years ago as a hybrid between *C. orientalis* and *C. grandiflora* (Douglas *et al.* 2015). It has by far the largest species ranges with an almost worldwide distribution owing to historical colonization in Eurasia and very recent human dispersal across other continents (Cornille *et al.* 2016). In Eurasia, three genetic clusters can be distinguished, likely corresponding to colonization events from Middle East (the probable centre of origin) to Europe and then to Eastern Asia. Across its expansion range, genetic diversity decreases and deleterious mutations have accumulated from Middle East to Eastern Asia (Cornille *et al.* 2016; Kryvokhyzha *et al.* In prep), in agreement with population genetic predictions on range expansion dynamics (Excoffier *et al.* 2009; Peischl *et al.* 2013; Peischl & Excoffier 2015).

In a previous study (Petrone-Mendoza *et al.* 2017), *C. rubella* has been shown to be more sensitive to competition than both *C. grandiflora* and *C. bursa-pastoris*, but no significant difference was observed between *C. grandiflora* and *C. bursa-pastoris*. However, this study focused only on Greek populations where the three species are found in sympatry. It was not representative of the whole species range of *C. bursa-pastoris* and one of the diploid progenitors of *C. bursa-pastoris*, *C. orientalis* was missing. Here, we performed a new competition experiment to compare the competitive ability

of *C. bursa-pastoris* with its two parental species, *C. grandiflora* and *C. orientalis*, and within *C. bursa-pastoris*, among populations across the species range. For completeness and comparison with Petrone-Mendoza et al. (Petrone-Mendoza *et al.* 2017) we also added *C. rubella*. Our working hypotheses were (i) that competitive ability of *C. bursa-pastoris* should be intermediate between *C. grandiflora* on one hand, and *C. rubella* and *C. orientalis* on the other hand, and (ii) that competitive ability should decline across range expansion within *C. bursa-pastoris*.

Material and methods

Studied species and sampling

We sampled the four *Capsella* species: *C. grandiflora* (the diploid outcrosser), *C. rubella* and *C. orientalis* (the two diploid selfers) and *C. bursa-pastoris* (the tetraploid selfer). One accession was initially sampled from each of the 62 populations of *C. bursa-pastoris* studied by Cornille et al. (Cornille *et al.* 2016), including 22 European, nine Middle Eastern, and 31 Chinese populations respectively. We added five populations from Central Asian, an area that was not sampled in Cornille et al. (Cornille *et al.* 2016) (Table S1). In addition, 15 to 35 accessions from each of the three other *Capsella* species sampled over their species range were used for interspecific comparisons, corresponding to 5 *C. orientalis*, 16 *C. rubella* and 9 *C. grandiflora* populations (Table S1).

A previous work (Petrone-Mendoza *et al.* 2017) has shown that the difference in sensitivity to competition was weakly affected by the nature of the competitor. As a result we used only one, non-*Capsella*, competitor species to simplify the experimental

design and ensure the same inter-specific competition for the four species. As in Petrone-Mendoza et al. (Petrone-Mendoza *et al.* 2017), we used *Matricaria chamomilla*, an annual Asteraceae species, which was found to co-occur with *Capsella* species in Greek populations and at least with *C. bursa-pastoris* in other European populations (personal observations). More generally, *Capsella* species and *M. chamomilla* have overlapping distributions (Europe and temperate Asia), and share their rosette growth pattern and weedy habits. We used commercial seeds for *M. chamomilla*, ensuring good germination and homogeneity among plants.

Experimental design

A competition experiment was performed in order to assess the competitive ability of all *Capsella* species. Each accession was tested with four replicates with and without competition in a complete random block design. For the "competition" treatment, one focal *Capsella* individual was sown in the middle of an 11cm x 11cm x 11cm pot, surrounded by four *M. chamomilla* competitors. For the "alone" treatment, focal individuals were sown without competitors.

30 seeds from each accession were first surface-sterilized. For stratification, seeds were sown in agar plates in darkness for 6 days at 4°C. Following sterilization and stratification, agar plates were moved to a growth chamber at 22 °C with 16h light and 8h darkness, under the light intensity of 130 lmol/m2/s for germination. After 5 days, germinated competitors were transferred to pots filled with standard culture soil and were placed randomly in two growth chambers (each growth chamber has two blocks) under the same temperature and light conditions as during the germination period. After recording the germination rates of each accession, focal species were sown five

days after the competitors in order to ensure strong enough competition against *Capsella* species. Accessions with less than eight germinated seeds (the minimum required to fulfill the design) were removed. Seedlings were watered daily for 2 weeks and every other day until the end of the experiment.

A series of fitness-related traits of focal individuals were measured during the experiment. Three weeks after the seedlings had been sown, two perpendicular diameters of the rosette were measured the first time and then again one week later. We used the product of these two diameters as a proxy for rosette surfaces (S1 and S2). From them, we calculated growth rate as the relative difference of rosette surface: (S2 – S1)/S1. When plants senesced, approximately 40 days after the beginning of flowering, the total number of flowers was counted for each focal individual. As in Petrone-Mendoza et al. (Petrone-Mendoza *et al.* 2017), we used flower numbers, rather than fruit or seed numbers, to allow comparison between the four species because *C. grandiflora*, which is self-incompatible, set almost no fruit due to the absence of pollinators in the growth chambers.

Genetic diversity data

Except for the five recently collected individuals from Central Asia, population structure of *C. bursa-pastoris* has been inferred using Genotyping By Sequencing (GBS) data in Cornille et al. (Cornille *et al.* 2016). In our analysis, we used the same SNP data to estimate genetic diversity. In brief, 253 accessions from the 62 populations throughout Europe, Middle East and Asia were genotyped by sequencing (GBS) using the *C. rubella* genome v1.0 (Slotte *et al.* 2013) as a reference for mapping. After checking for sequencing reliability, filtering against sequencing errors and fixed heterozygote

sites, 4274 SNPs were available for further analysis (see Cornille *et al.* 2016 for details). Mean pairwise nucleotide differences (π) (Nei 1978) were obtained with arlsumstat (Excoffier & Lischer 2010) for each sampling site with 0.5% missing data allowed. π values were corrected for missing data and divided by 64-bp (GBS markers' length) to obtain the mean pairwise nucleotide differences per site, which we used as a proxy for local genetic drift. Genetic diversity data are given in Table S2.

Data analysis

Data were analysed with generalized linear mixed models in R version 3.3.2 (R Development Core Team 2011). For all variables, block, species, treatment and species x treatment interaction were included as fixed effects and accessions as random effect. For analyses within *C. bursa-pastoris* we used the area of origin as a factor instead of species. For linear models, the significance of the different factors was tested with type III analyses of variance using the *anova* function. For generalized linear models we performed analyses of deviance using the *Anova* function of the *car* package using the type III ANOVA option (Fox & Weisberg 2011). For linear models, random effects where tested by likelihood ratio tests using the *rand* function of the *lmerTest* package (Kuznetsova *et al.* 2016). For generalized linear models we also ran their equivalent models without random effects and compared the likelihood of the models with the *anova* function.

Rosette surface and growth rate were analysed using a mixed linear model fitted by maximum likelihood with the *lmer* function of the *lme4* package (Bates *et al.* 2015). The flower number distribution was bimodal with a mode at 0 and another around 1000 (see results). Thus it was analysed in two steps. First, we analysed the proportion

of flowering plants with a binomial model and a logit link using the *glmer* function of the R package *lme4* (Bates *et al.* 2015). Dead plants were included in the non-flowering category. Second, we excluded plants that did not flower and analysed flower number with a negative binomial model and a log link with the *glmmadmb* function of the *glmmADMB* package (Fournier *et al.* 2012; Skaug *et al.* 2013).

For the comparison among species, we were specifically interested in the species*treatment interaction term to determine whether the effect of competition differed among species. For comparison within *C. bursa-pastoris*, we were interested in the area*treatment interaction term to determine whether the effect of competition differed across the expansion range. If significant, we then analysed a competition index, as defined in Petrone-Mendoza et al. (Petrone-Mendoza *et al.* 2017), to get a more direct estimate and more intuitive interpretation of the result:

$$I_c = \frac{W_{competition}}{W_{alone}}$$

where W_{alone} and $W_{competition}$ are the fitness-related trait of the focal accession without and with competition, respectively. The lower I_c, the more sensitive to competition the accession is. I_c was computed separately for each accession in the four blocks. We analysed the I_c indices with mixed linear models using the *lmer* function of the *lme4* package (Bates *et al.* 2015) with block and species or area as fixed effects and accession as random effect. Mean effects were estimated with the *lsmean* function of the *lmerTest* package (Kuznetsova *et al.* 2016) and differences between levels were tested using the *contrast* function of the *contrast* package, with False Discovery Rate (FDR) correction for multiple testing.

Finally, we tested whether differences in I_c could be explained by differences in genetic diversity among populations, with the prediction that I_c should increase with genetic diversity. To do so, we ran a simple linear model with block and Pi as fixed effects. As each population is represented by its genetic diversity we did not include populations as random effect in this model. The five accessions from central Asia were not included in this analysis because we lack genetic data for them.

Results

After having discarded accessions with too few seeds, the final data set contains 13 accessions of *C. grandiflora*, 33 of *C. rubella*, 19 of *C. orientalis* and 49 of *C. bursa-pastoris*, distributed in the four geographic regions as follows: nine from Middle east, 17 from Europe – including eastern Russian populations that belong to same genetic cluster, see (Cornille *et al.* 2016) – five from central Asia – without genetic characterization – and 18 from China (Table 1). During the experiment survival rates were very high (> 90%) for all species and most plants flowered (Table 1).

First we compared the four species, considering *C. bursa-pastoris* as a single unit. Rosette surfaces at the two times, but not growth rate, were significantly and negatively affected by competition (Table 2) but the effect is modest and competition only reduced rosette surface by around 5% (Figure 1). Moreover, the four species did not differ in their sensitivity to competition (the species x treatment effects were not significant, Table 2 and Figure 1) and there is no species effect on the competition index (Figure 1 and ANOVA in Table S3). On the contrary we detected a strong effect of competition on flowers number and the four species differed in their sensitivity to competition (Table

3). This is confirmed by the analyses of the competition index (Figure 2 and ANOVA in Table S3). *C. orientalis* and *C. rubella* are the most affected by competition with a reduction in flowers number by a factor two: I_c = 0.52 (0.47 – 0.56) and 0.49 (0.45 – 0.53) whereas *C. grandiflora* is the least affected: I_c = 0.75 (0.69 – 0.80). *C. bursa-pastoris* is intermediate with I_c = 0.65 (0.62 – 0.68).

Then we analyzed the four geographic areas within *C. bursa-pastoris*. We also detected a negative effect of competition on rosette surfaces but not on growth rate (Table 4). In addition we found a significant area x treatment effect for the second measure of rosette surface (Table 4), which is confirmed by the analysis of the competition index (Figure 3 and ANOVA in Table S4). However the effect of competition is weak with I_c varying from 0.85 (0.81 – 0.90) in Middle East to 0.99 (0.95 – 1.03) in China, Central Asia and Europe being intermediate with 0.93 (0.85 – 1.00) and 0.95 (0.91 – 0.98), respectively. As for comparison among species, competition also had a strong and differential impact among geographic areas on flowers number (Table 5 and Figure 4). However, the pattern is almost reverse to rosette surface. China is the most sensitive to competition with I_c = 0.55 (0.51 – 0.59) and Middle East the least, I_c = 0.76 (0.71 – 0.82), Central Asia and Europe being intermediate with 0.68 (0.60 – 0.77) and 0.69 (0.65 – 0.73), respectively (Figure 4 and ANOVA in Table S6). This apparent contradiction can be partly understood by looking at the correlation between rosette surface and flowers number. Flowers number is significantly and positively correlated with rosette surface but this is not sufficient to explain the difference between the "alone" and "competition" treatments (Figure 5). The area x treatment interaction effect for flowers number is even more significant when rosette surface is added as a

covariable in the model (Table 5). This suggests that rosette surface is not a sufficient proxy for fitness. Note that this was also observed for the three other species (Table S7).

We then tested whether difference in genetic diversity could explain the observed difference among geographic areas. Genetic diversity had no significant effect on I_c for rosette surface when it is used instead of population and area effects (not shown). On the contrary, I_c for flowers number is positively and significantly correlated with genetic diversity (Figure 6). However, the area effect remained significant after removing the genetic diversity effect (Type I ANOVA) and it overwhelmed the genetic diversity effect when they were tested jointly (Type III ANOVA) (Table S8).

Discussion

It has been recognized for a long time that the evolution of self-fertilization is often associated with a series of ecological traits, such as colonizing ability, annuality or weediness. Recently, such ideas have been tested more formally with a meta-analysis, using the length of Grime's CSR theory of ecological strategies: selfing was found associated with the ruderal habit and lower competiveness (Munoz *et al.* 2016). To better understand how competitive ability may vary with mating system we compared the effect of competition in four *Capsella* species differing by their mating system and ploidy level. In the tetraploid selfer, *C. bursa-pastoris*, we also characterized the effect of competition across the expansion range of the species. Rapid range expansion thanks to good colonizing abilities is also an ecological attribute of selfing species (Randle *et al.* 2009; Grossenbacher *et al.* 2015), and has been suggested to be negatively associated with competitiveness (e.g. Burton *et al.* 2010).

Variation in competitive ability within and among *Capsella* species

Our main findings are in agreement with theoretical predictions. Using the number of flowers as a proxy for fitness, the outcrossing, *C. grandiflora*, was the least sensitive to competition while the two diploid selfers, *C. orientalis* and *C. rubella*, were the most sensitive (Figure 2). The tetraploid selfer, *C. bursa-pastoris*, was intermediate between its two putative parents (Figure 2), and within the species, the sensitivity to competition increased from the possible region of origin to the expansion front (Figure 4). In a previous experiment, *C. rubella* was already found as the most sensitive to competition but no significant difference was found between *C. grandiflora* and *C. rubella*. However, in this experiment only Greek accessions were used for *C. bursa-pastoris*, mainly corresponding to the Middle East genetic cluster (Cornille *et al.* 2016). Here also, Middle East accessions did not significantly differ from *C. grandiflora* (I_c = 0.76 [0.71 – 0.82], and I_c = 0.75 [0.69 – 0.80], respectively, Figures 1 and 3). The global difference between the two species was mainly attributable to the rest of the species range.

During the course of the experiment we also measured the surface of the rosette of the plants at two different times. While this trait is affected by competition, the global effect was rather weak and did not vary among species. Within *C. bursa-pastoris*, however, the effect of competition decreased across the species range, contrary to expectations and to what was observed for flower production. A possible caveat is that rosette surface estimates were less precise than flowers number estimates. Alternatively, this could reflect that rosette surface is not a very good proxy for fitness, although it is positively correlated with flowers number (Figure 5). It also suggests that

access to light was not the main driver of competition in the experiment as difference in rosette surface could not explain the difference in flowers number between treatments (Figure 5). In the experiment, the strongest part of competition likely occurred at the root levels as we chose rather small pots for this reason. Moreover, for both treatments, the row values of rosette surface corresponds to the hypothesized qualities of the four geographic area, with decreasing values from Middle East to China the lowest (Figure 3).

The underlying causes of the observed pattern cannot be established from this sole experiment and the results are in agreement with two main theoretical hypotheses: the colonization/competition trade-off and the mutation load hypotheses. If transition to selfing is associated with transition to a more ruderal strategy and colonization of low competitive habitats, relaxed selection on competitive traits could explain the stronger effect of competition on the three selfing species. The same rationale would apply for the increased sensitivity to competition across the expansion range in *C. bursa-pastoris*. Alternatively, competitive ability could be affected by the mutation load. In agreement with theoretical predictions on the genetic effects of selfing, selection against deleterious mutations is relaxed in the three selfing species compared to the outcrossing one (Brandvain *et al.* 2013; Slotte *et al.* 2013; Douglas *et al.* 2015). Moreover, within *C. bursa-pastoris*, Chinese populations corresponding to the expansion front showed evidence of a higher load than European and Middle East populations (Kryvokhyzha *et al.* In prep). So both among and within species, the mutation load is negatively correlated with competitive ability. As in other studies (reviewed in te Beest *et al.* 2012), we also observed a difference between the diploid and the tetraploid selfers. This is also in agreement with the mutation load theory with a masking effect of

deleterious mutations in polyploids (Comai 2005). Alternatively, it could be a direct consequence of increased cell size induced by genome doubling (Comai 2005; te Beest *et al.* 2012). The link with the colonization/competition trade-off hypothesis is less clear but we can speculate that gene redundancy could more easily reduce the pleiotropy of genes affecting both colonization and competition traits.

Implication for the long-term evolution of selfing species

Whatever the underlying causes, the relationship between mating system and competitiveness suggests new implications for the long-term evolution of selfing species. In particular, it could help resolving the paradox of selfing species that appear ecologically and demographically successful in the short term (Grossenbacher *et al.* 2015), but an evolutionary dead-end in the longer term (Stebbins 1957; Igic & Busch 2013; Wright *et al.* 2013). Some ecological conditions, such as disturbed, temporary or newly opened habitats, can both favor the evolution of selfing through selection for reproductive assurance, and correspond to weakly competitive environments that would allow their persistence despite poor competitive ability. Interestingly, selection is expected to be softer when interspecific competition is low. This is expected to allow mutations to build up without demographic consequences (Agrawal 2010; Agrawal & Whitlock 2012). However, selfing species would be trapped into these weakly competitive habitats. Increase in competition, for example during succession, would increase extinction risk by competitive exclusion, especially because the accumulated genetic load would have stronger demographic impact than under the initially less competitive conditions (Agrawal 2010; Agrawal & Whitlock 2012).

A recent meta-analysis suggested that selfing species could experience diminished niche breadth over time despite geographic expansion (Park *et al.* 2017). The authors suggested that it could be due to the lack of long-term adaptive potential and accumulation of deleterious mutations. Reduced competitive ability could also prevent their establishment in new habitats. The scenario proposed by Park at al. (Park *et al.* 2017) is related to ours where the decrease in available habitats over time would finally reduce geographic range after initial range expansion. This would provide an ecological scenario for the higher extinction rate in selfers underlying the dead-hypothesis and the maintenance of outcrossing species through species selection (Goldberg *et al.* 2010; Igic & Busch 2013). More generally, it could also contribute to explain how selfing and asexual lineages could "senesce" in diversification rates, as recently proposed (Ho & Agrawal 2017). If species derived from outcrossing ancestors are rapidly trapped into restricted non-competitive habitats, subsequent newly formed selfing species (from already-selfing ancestors) would also inherit restricted ecological niches without beneficing from higher reproductive assurance compared to ancestors.

Finally, under the global scenario proposed above, polyploidy could buffer the negative effect of selfing and delay the extinction risk. The association between selfing and polyploidy (Barringer 2007; Robertson *et al.* 2011) could be due both to the facilitation of the shift to polyploidy by selfing (Rodriguez 1996; Rausch & Morgan 2005) and to the reduction of extinction risk in selfers by polyploidy.

Conclusion

Our main results are in agreement with theoretical predictions on the effects of mating system (including the range expansion dynamics) and ploidy level on competitive abilities. They are also congruent with a meta-analysis showing association between outcrossing and competitiveness (Munoz *et al.* 2016). However, we are not aware of other direct comparison among close species and our results could be only specific to the *Capsella* genus. Thus, they remain to be confirmed at a larger scale in different species. The role of the mutation load during range expansion (Peischl *et al.* 2013; Peischl & Excoffier 2015; Peischl *et al.* 2015; Gilbert *et al.* 2017) and its interaction with competitive conditions (Agrawal 2010; Agrawal & Whitlock 2012) have been well developed from a theoretical view point. Our results could fit with these appealing hypotheses but more direct relationships between variation in load and variation in fitness in various competitive conditions remains to be tested in natural populations. Combining genetic, ecological and demographical approaches has already been advocated to understand the transition from outcrossing to selfing (Cheptou 2007; Cheptou & Schoen 2007). We suggest that this should also be a promising approach to a better understanding of the long-term fate of selfing species.

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Tables

Table 1: characteristics of the dataset

Species		# accessions used	Survival rate (%)	Flowering rate (%)
C. grandiflora		13	96.8	87.5
C. rubella		33	92.1	78.4
C. orientalis		19	100	100
C. bursa-pastoris	Total	49	91.1	90.1
	Middle East	9	100	100
	Europe	17	95.6	95.6
	Central Asia	5	90.6	87.5
	China	18	82.6	82.6

Table 2. Analyses of variance for vegetative traits for the comparison among species

		Rosette surface 1			Rosette surface 2			Growth rate		
	DoF	SS	F	p-value	SS	F	p-value	SS	F	p-value
block	3	290.30	91.43	**< 2e-16**	157.75	47.67	**<2e-16**	1.43	21.96	**1.27e-13**
species	3	7.32	2.30	0.081	6.99	2.11	0.102	0.06	0.84	0.472
treatment	1	77.40	73.13	**< 2e-16**	128.94	116.90	**<2e-16**	0.004	0.18	0.671
species*treatment	3	5.89	1.85	0.136	6.48	1.96	0.119	0.09	1.33	0.262
		LR		p-value	LR		p-value	LR		p-value
accession (random)	1	378		**< 2e-16**	394		**< 2e-16**	128		**< 2e-16**

Table 3. Analyses of deviance for reproductive traits for the comparison among species

		Proportion of flowering plants		Number of flowers	
	DoF	LR	p-value	LR	p-value
block	3	15.71	**0.001**	46.65	**4.12e-10**
species	3	5.65	0.130	200.87	**< 2e-16**
treatment	1	0.00	0.999	377.87	**< 2e-16**
species*treatment	3	1.04	0.792	94.86	**< 2e-16**
Accession (random)	1	40.89	**1.62e-10**	389.04	**< 2e-16**

Table 4. Analyses of variance for vegetative traits for the comparison among *C. bursa-pastoris* geographic areas

		Rosette surface 1			Rosette surface 2			Growth rate		
	DoF	SS	F	p-value	SS	F	p-value	SS	F	p-value
block	3	179.31	65.53	**< 2e-16**	93.41	27.51	**8.88e-16**	0.78	13.31	**3.53e-08**
area	3	18.61	6.80	**0.0007**	43.75	12.88	**2.65e-06**	0.08	1.42	0.249
treatment	1	18.82	20.63	**8.02e-06**	40.84	36.09	**5.36e-09**	0.02	0.89	0.347
area*treatment	3	6.43	2.35	0.073	26.57	7.83	**4.76e-05**	0.07	1.14	0.334
		LR		p-value	LR		p-value	LR		p-value
accession (random)	1	185		**< 2e-16**	146		**< 2e-16**	61.1		5e-15

Table 5. Analyses of deviance for reproductive traits for the comparison among *C. bursa-pastoris* geographic areas

		Proportion of flowering plants		Number of flowers		Number of flowers	
	DoF	LR	p-value	LR	p-value	LR	p-value
block	3	16.07	**0.0011**	26.85	**6.33e-06**	10.06	**0.018**
area	3	1.43	0.698	1.03	0.794	1.00	0.800
treatment	1	0.45	0.504	25.29	**4.94e-07**	21.36	**3.80e-06**
area*treatment	3	1.63	0.652	9.74	**0.021**	12.81	**0.005**
Accession (random)	1	13.35	**0.0002**	175	**< 2e-16**	152	**< 2e-16**
Rosette surface 2	1	/	/	/	/	13.67	**0.0002**

Figure 1: Rosette surface (A) and least-squares mean (± CI) of corresponding competition Indices (B) for the four species.

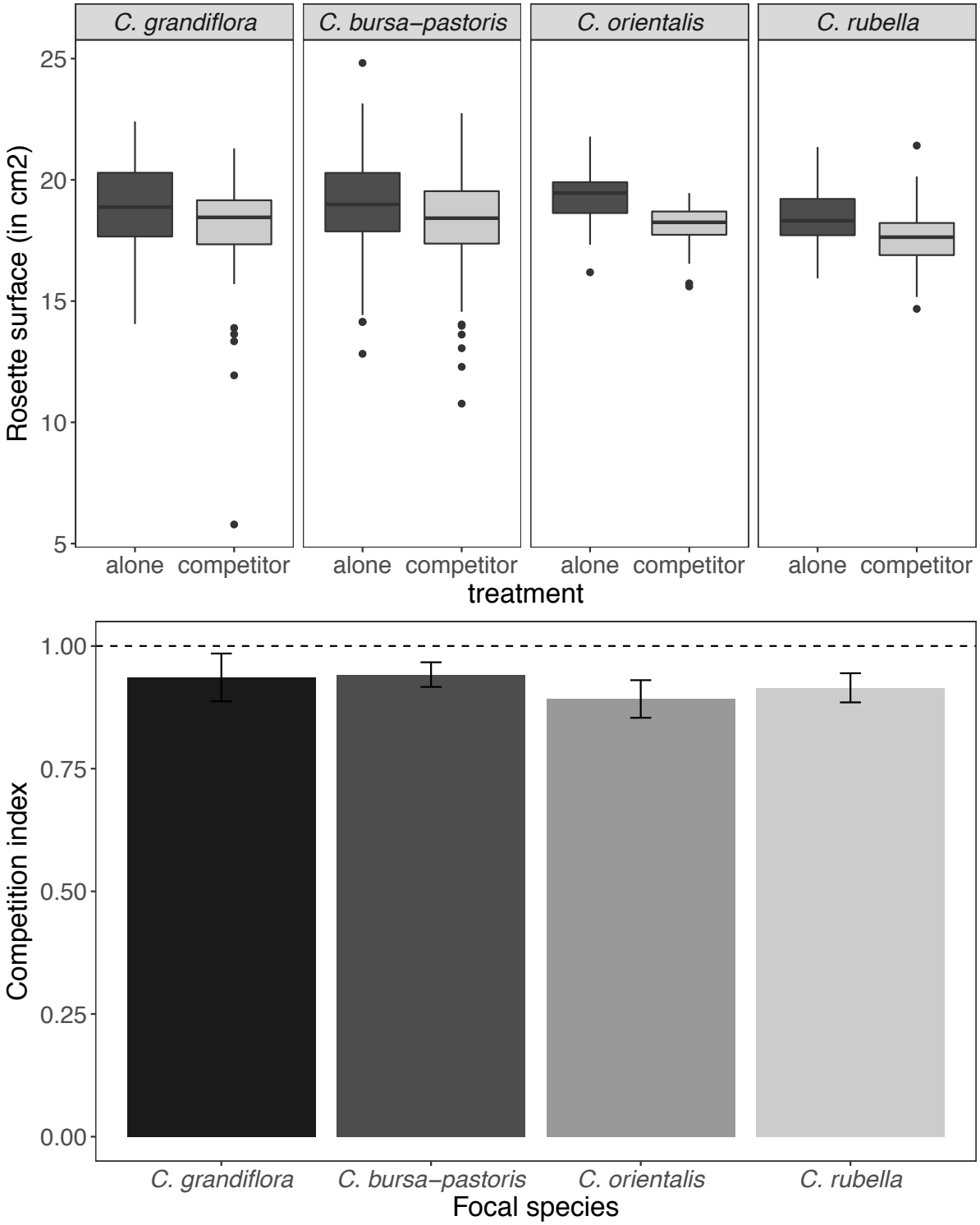

Figure 2: Flowers number (A) and least-squares mean (± CI) of corresponding competition Indices (B) for the four species.

In (B), different letters indicate significantly different estimates.

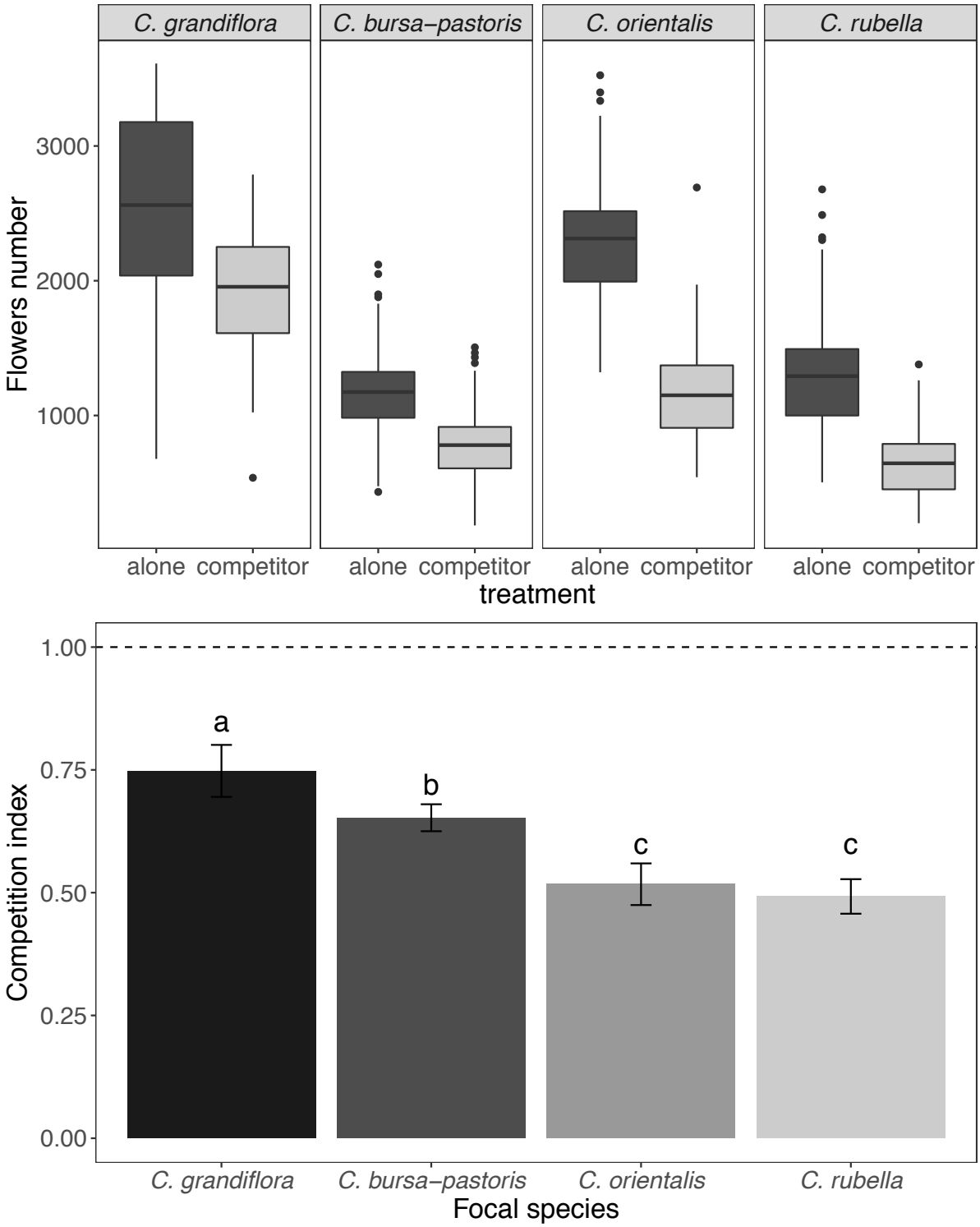

Figure 3: Rosette surface (A) and least-squares mean (± CI) of corresponding competition Indices (B) for the four areas of *Capsella bursa-pastoris*.

In (B), different letters indicate significantly different estimates.

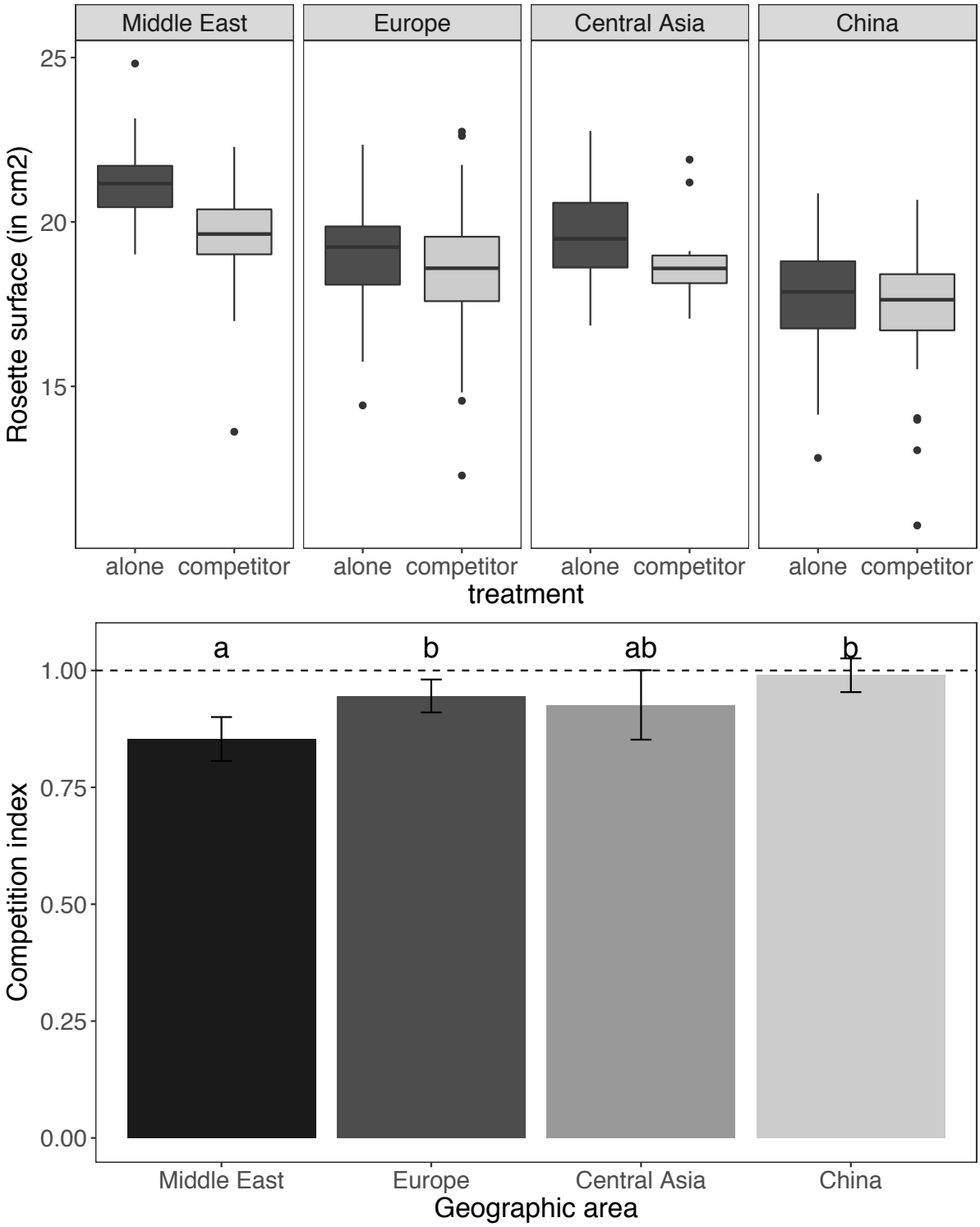

Figure 4: Numbers of flowers (A) and least-squares mean (± CI) of corresponding competition Indices (B) for the four areas of *Capsella bursa-pastoris*.

In (B), different letters indicate significantly different estimates.

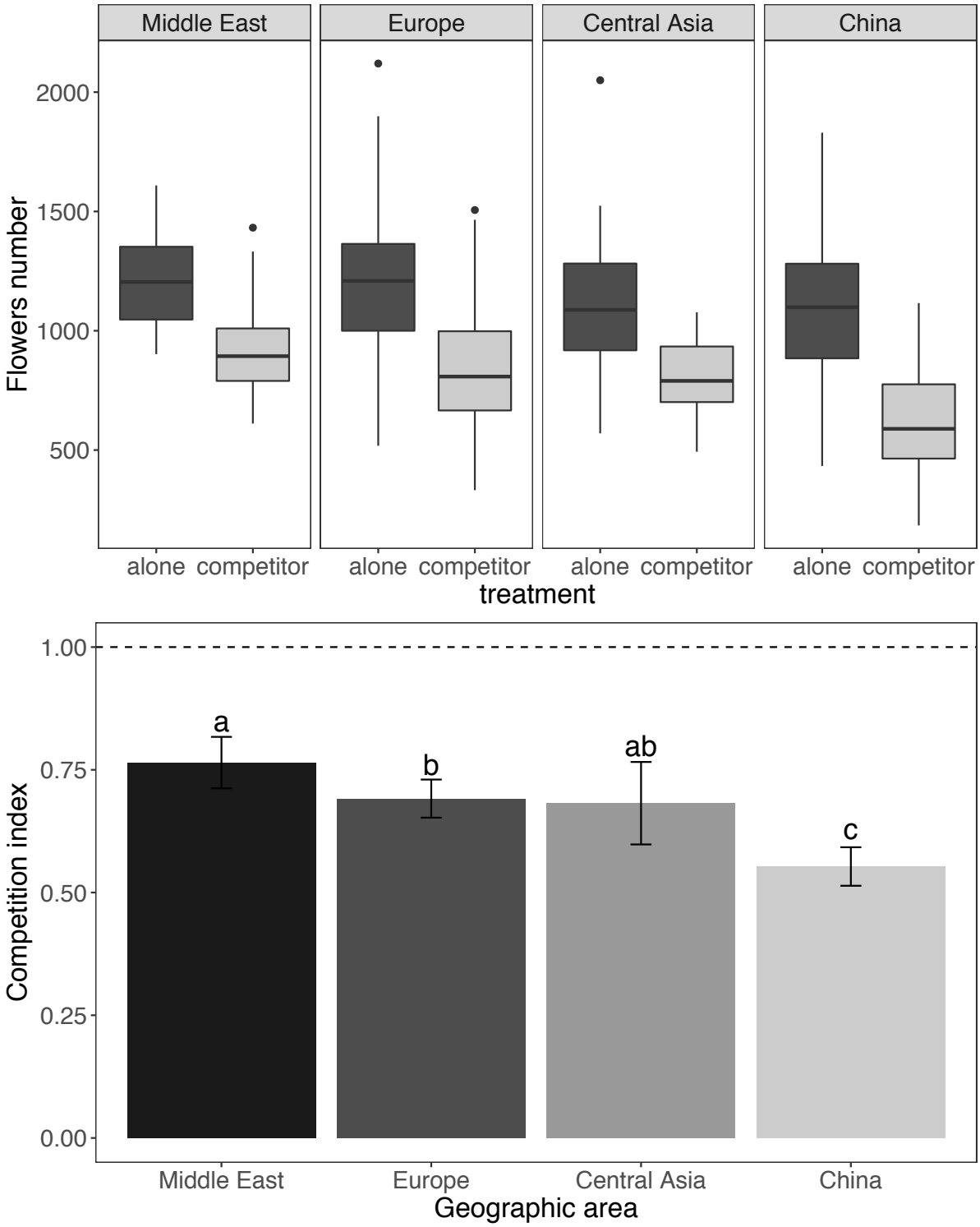

Figure 5: Correlation between rosette surface and flowers number for the two treatments in *C. bursa-pastoris*

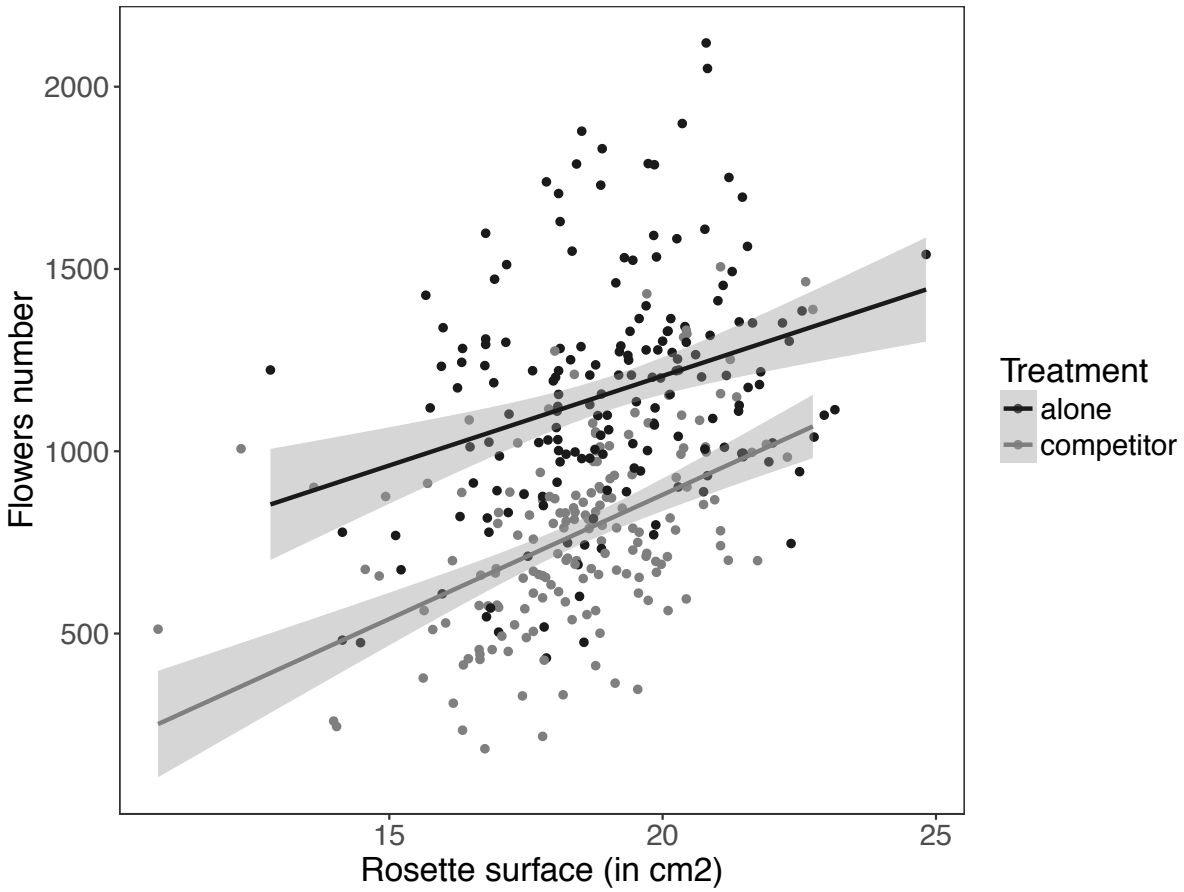
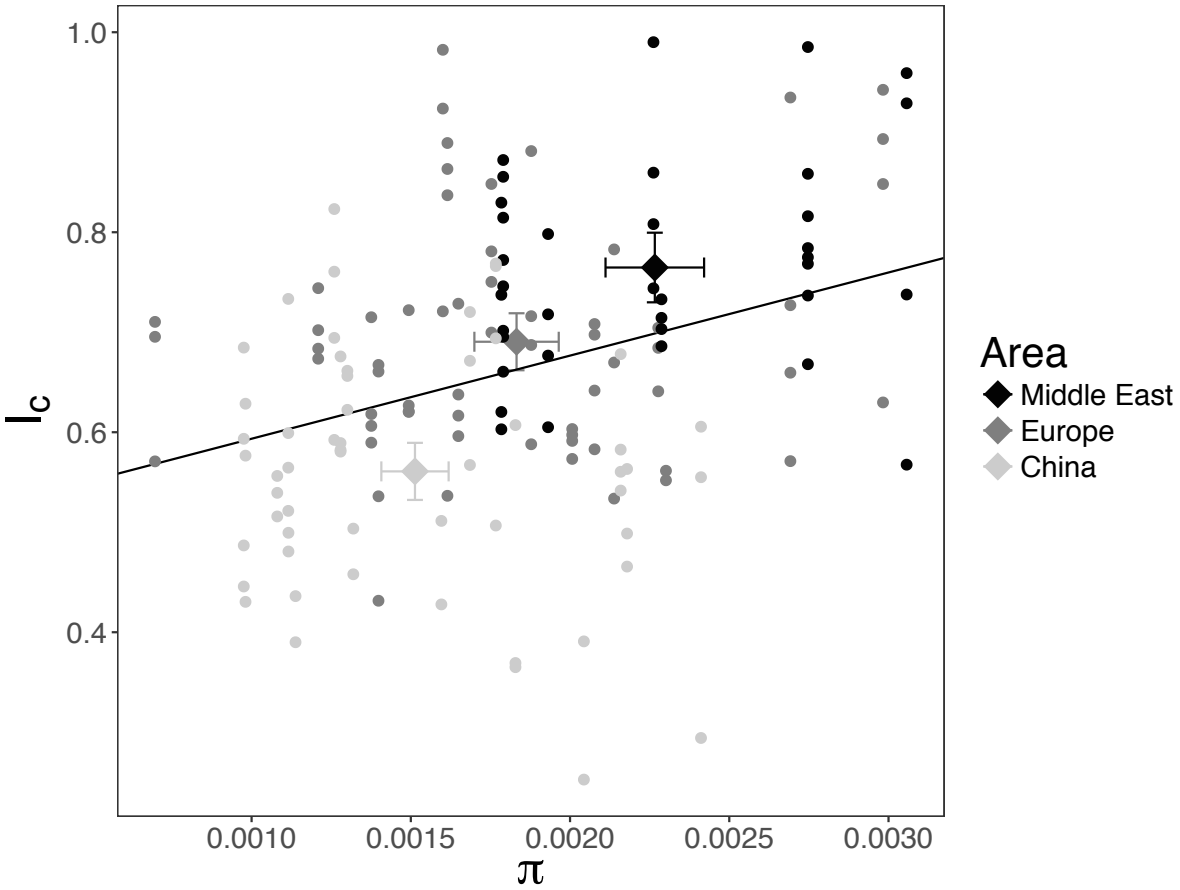
Figure 6: Competition indices (I_c) as a function of genetic diversity for the geographic areas within *Capsella bursa-pastoris*. The means +/- se per geographic area of the two variables are also plotted with the global regression line.