

Population structure effect on reproduction of a rare plant: beyond population size effect

Arantzazu L. Luzuriaga, Adrián Escudero, María José Albert, and Luis Giménez-Benavides

Abstract: Several studies have evaluated the effect of population size on plant reproductive output, but there are few studies concerning the effect of other population structure variables on plant reproduction. In this study, we went beyond population size effect and we tested the effect of some population spatial variables such as plant density, plant aggregation, population structure, and population identity on plant reproductive success and fitness in large populations (>1000 individuals) of *Centaurea hyssopifolia* Vahl., an endemic species of central Spain. We analysed the reproductive output (capitula, flowers, and seeds) and another two components of plant fitness (seed mass and germinability) in 350 individuals from seven populations in 2002 and 2003. All populations were similar in size but differed in other population spatial characteristics (density, plant aggregation pattern, population plant size structure). We used Morisita's aggregation index to characterize the level of plant aggregation within populations. Population plant size structure variable determined the proportion of different sized plants in a population. We used generalized linear mixed models to model the contribution of these population spatial variables to several reproductive parameters. Our results showed that whereas the aggregation index exerted a positive control on plant seed set, plant density had a negative effect, and plant population size structure did not significantly influence any response variable. Density only exerted a negative effect on seed set. On the contrary, plant aggregation had a positive effect. Seed mass was also larger in more aggregated populations, although no differences in seed germinability were observed. We detected the effect of population attributes on reproduction in terms of seed set, but we did not detect any effect on other overall reproductive variables measured at plant level. To sum up, our results showed that if population fragments were large enough, spatial population attributes became crucial factors for plant reproductive output and fitness. These easily measurable population variables may improve the conservation management of rare plant species.

Key words: Astereaceae, density, germinability, plant aggregation, plant size structure, reproductive output.

Résumé : Plusieurs études ont évalué l'effet de la dimension des populations sur le rendement reproductif des plantes, mais peu d'études concernent l'effet des autres variables de la structure des populations sur la reproduction des plantes. Dans cette étude, les auteurs vont au-delà de l'effet de la dimension des populations. Ils évaluent les effets de certaines variables spatiales des populations, telles que la densité des plantes, l'agrégation des plantes, la structure de la population et l'identité de la population sur le succès reproductif des plantes et l'adaptation, dans de grandes populations (>1000 individus) du *Centaurea hyssopifolia* Vahl., une espèce endémique du centre de l'Espagne. Ils ont analysé le rendement reproductif (capitules, fleurs et graines) et deux autres composantes de l'adaptation (dimension des graines et pouvoir germinatif), chez 350 individus provenant de sept populations, en 2002 et 2003. Toutes les populations étaient semblables en dimension, mais différaient quant à d'autres caractéristiques spatiales de la population (densité, patron d'agrégation des plantes, structure de la dimension des plantes dans la population). Les auteurs ont utilisé l'index d'agrégation de Morisita, pour caractériser le degré d'agrégation des plantes au sein des populations. La variable de la structure de la dimension des plantes de la population détermine la proportion des différentes dimensions de plantes dans la population. On a utilisé les modèles linéaires mixtes généralisés pour modéliser la contribution de ces variables spatiales des populations à plusieurs paramètres reproductifs. Les résultats montrent que si l'index d'agrégation exerce un contrôle positif sur la mise à graine des plantes, la densité des plantes a un effet négatif, et la structure de la dimension des plantes de la population n'influence significativement aucune variable de réaction. La densité n'exerce une influence négative que sur la mise à graine. Au contraire l'agrégation des plantes a un effet positif. La masse des graines est également plus importante dans les populations plus fortement agrégées, bien qu'il n'y ait pas de différences dans le pouvoir germinatif des graines. On détecte des effets des attributs de la population sur la reproduction, en termes de mise à graine, mais on ne perçoit pas d'effet sur l'ensemble des autres variables reproductives, mesurée à l'échelle de la plante. En résumé, les résultats montrent que si les fragments de population sont suffisamment grands, les attributs spatiaux de la population deviennent des facteurs cruciaux pour l'intensité de la reproduction et l'adaptation. Ces variables de populations, facilement mesurables, pourraient améliorer l'aménagement en vue de la conservation d'espèces rares.

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Mots clés : Asteraceae, densité, pouvoir germinatif, agrégation des plantes, structure de la dimension des plantes, intensité reproductive.

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Introduction

The effects of population size and plant density on plant reproduction have received great attention in habitat fragmentation contexts (Agren 1996; Fischer and Matthies 1998; Hackney and McGraw 2001; Mustajärvi et al. 2001; Brys et al. 2004), as well as other population characteristics such as isolation degree (Morgan 1999; Schulke and Waser 2001; Jacquemyn et al. 2002) and habitat quality (Hobbs and Yates 2003; Brys et al. 2004). Surprisingly, other spatial and structural aspects of populations, such as the level of plant aggregation and plant size structure have been poorly studied in this context. Plant size structure provides information about the proportion of different-sized plants in a population. Consequently, plant size structure is also expected to affect pollinator attraction, because the more abundant are large plants in the population, the more attractive the population would be for pollinators. Some studies evaluated aggregation of individuals measured as the nearness of neighbouring plants to a target individual and they observed that aggregation significantly affected visitation rates and foraging behaviour of pollinators (Roll et al. 1997). However, we believe that plant aggregation measured at population level also exerts meaningful effects on pollination and consequently on plant reproduction; nevertheless the effect of aggregation measured at population level has only been partially explored using a surrogate such as plant density (Van Treuren et al. 1993).

We hypothesized that reproductive output and other fitness components of plants may be affected not only by some population parameters linked to the biogeographical theory of islands (i.e., fragment size and connectivity) but also by other population structure characteristics such as plant density, aggregation pattern (Morisita's index), and plant size structure. These population-level characteristics would be important variables to consider in the conservation biology of species, since these factors may potentially be at least as important as population size itself (Ashman et al. 2004; Knight 2004) and are easily measurable. We modelled plant reproductive output and other fitness components (seed mass and germination) of the rare plant species with the above plant population predictors. We considered seven populations with relatively high but similar size (>1000 individuals) of an endemic and rare gypsophyte of central Spain, *Centaurea hyssopifolia* Vahl. (Asteraceae). Because seed production and other important fitness components are usually related to plant size (Colas et al. 2001), we partialled out the effect of plant-level variables such as plant size and total inflorescences (capitula) production. Reproduction involves processes that occur at different spatial scales in a hierarchical fashion (e.g., capitula within plant, plants within population). Nevertheless, empirical studies have mostly focused on one particular level (plant or population), and if more than one level was considered simultaneously, they

rarely considered an explicit multiscale perspective. To overcome such problems and to properly assign the influence of each variable at each level, we conducted GLMMs (generalized linear mixed models) (Buckley et al. 2003). In this paper we addressed the following questions: (1) What is the importance of the three population-level variables studied, that is, plant density, plant aggregation, and plant size structure on reproduction and fitness of *Centaurea hyssopifolia*? (2) Is the expected relationship between plant size and plant fitness independent of the aggregation of plants within populations? (3) Do the interannual environmental variability between both study years (2002 and 2003) affect the relative importance of these parameters?

Materials and methods

Study species and sites

Centaurea hyssopifolia is an endemic cushion shrub inhabiting gypsum outcrops of semiarid central Spain. Flowering plants reach a height of 10–50 cm, a diameter of 10–87 cm, and produce 2–560 capitula. Each capitulum produces 21 flowers on average (minimum = 0 flowers to maximum = 49 flowers). Flowering period extends from late May to late July with the highest flowering intensity in mid-June (A.L. Luzuriaga, personal observation). Preliminary studies suggest that *C. hyssopifolia* is a self-incompatible species with entomophilous pollination (A.L. Luzuriaga, unpublished data), similarly to other *Centaurea* species such as *C. diffusa* Lam. and *C. maculosa* Lam. (Harrod and Taylor 1995).

Centaurea hyssopifolia, together with other gypsophytes such as *Helianthemum squamatum* (L.) Dum. Cours. and *Lepidium subulatum* L., constitute the dominant species of the dwarf shrub plant communities of the Iberian gypsum habitats. Gypsum plant communities form dense patches interspersed on a bare crust surface covered by lichens. Total cover of perennial plants is usually below 30%. Mediterranean gypsum ecosystems are considered among the most threatened habitats in the Mediterranean Basin (Gómez-Campo 1987; European Community 1992; Mota et al. 2003).

Human activities have fragmented gypsum shrub communities, and consequently *Centaurea hyssopifolia* populations have also suffered strong fragmentation. In this study, we selected seven populations of more than 1000 individuals. These populations were located on massive gypsum outcrops in the southeastern quarter of the Madrid province (Spain) and laid within a rectangular area limited by 40°05' to the south, 40°15' to the north, 3°15' to the east and 3°35' to the west. Average rainfall is 425 mm·m⁻² with no appreciable differences among sites. The nearest populations were 5 km apart, and the farthest populations were 30 km apart. They were selected to cover the distribution range of the

Table 1. Mean values, standard deviations (SD), and ranges of reproductive and fitness variables measured in *Centaurea hyssopifolia* in 2002 ($n = 350$ plants and 2591 capitula).

	Per capitulum		Per plant	
	Mean \pm SD	Range	Mean \pm SD	Range
Flower number	21.1 \pm 6.5	0–49	2073.3 \pm 2096	122–14490
Total viable seeds	7.2 \pm 6.1	0–32	692.3 \pm 810.3	0–5635
Seed set (proportion)	0.33 \pm 0.27	0–1	0.33 \pm 0.16	0–0.75
Capitulum number	—	—	95.5 \pm 85	7–492
Germination (%)	—	—	0.44 \pm 0.26	0–100
Seed mass (mg)	—	—	2.2 \pm 0.78	0.5–5.6

species. Field studies were carried out in the seven populations during 2002 and also in three of them in 2003.

Data collection

We counted every individual (flowering and vegetative) in 10 randomly distributed square replicates of 25 m², to measure plant density at each population. Maximum rosette diameter of every individual within random plots was measured up to 100 randomly selected individuals per population, to estimate population plant size structure as a measure of age structure. Subsequently, the ratios of individuals above and below the size mode were calculated. When this ratio was below 1, small-sized individuals were dominant in the population. We estimated aggregation among plants (i.e., spatial pattern) from the 10 replicates per population with the Morisita index (Morisita 1959). This is a contagion index based on the variance to mean ratio, which is theoretically independent of the mean size, density, and the number of quadrats: $I = 0$ indicates a random distribution, $I > 0$ indicates an aggregate distribution, and $I < 0$ indicates a uniform distribution.

Fifty plants were randomly selected within each population. Plant size and total number of capitula per plant were recorded. Ten mature and closed capitula per plant were collected at random, or every single capitulum when the number was below this threshold. Capitula were collected at the fruiting peak in late June in 2002 and 2003 with no more than 10 d of delay among populations. Plants were randomly selected in both years.

Capitula were dissected in the laboratory, and the viable, aborted, and parasitized seeds per capitulum were counted. The sum of the three categories was calculated as an estimate of the total flower number produced per capitulum, and seed set at capitulum level was the percentage of viable seeds out of the total flower number. Average values of flower number, viable seeds, aborted seeds, and seed set per capitulum were used to estimate total flower number, viable seeds, aborted seeds, and seed set per plant after multiplying by total capitula.

Individual seed mass (5 seeds per plant) and mean germination percentage per plant (50 seeds per plant) were measured as components of plant fitness. Seeds were weighted in a COBOS AX-120 balance, with precision of 0.1 mg. For the germination assays, two samples of 25 seeds per plant were placed on two sheets of filter paper (Whatman No. 40) in 9 cm Petri dishes. Petri dishes were located at random in germination chambers (Hotcold-GL) at 20 °C, which is

the optimum germination temperature for the species (Escudero et al. 1997). Incubation of seeds took place under a 16 h light : 8 h dark photoperiod, and an irradiance of 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was provided by cool white fluorescent tubes (OS-RAM L 58W/20). We kept the filter papers soaked throughout the 3-week assay period, and seeds with radicle emergence were counted and removed from the Petri dishes at 3–4 d intervals. Dish location in the chamber was periodically changed. Both subsamples of seeds per plant were considered together for the germination analysis.

Statistical analyses

Our data had an unbalanced and hierarchical structure, with capitula nested within plants and plants nested within populations. Hierarchical data structure implies correlation between data points at different scales, inflating the error degrees of freedom (df), and increasing the chance of type I error. To overcome these problems, data were analysed with a multilevel approach considering plants and populations as random factors and applying mixed modelling. Since our response variables clearly depart from the assumption of normality, we used generalized linear mixed models (GLMM). In GLMMs, the response variable, which is a (link) function from the original data, is a linear combination of fixed and random effects (see Littell et al. 1996). Depending on the error distribution of each response variable, we set different error distributions with the appropriate link function. A binomial estimator, using a “logit” link function and setting the variance to “mean (1-mean)”, was applied when the distribution of the response variable was a probability ranging from 0 to 1 (i.e., seed set, percentage of aborted seeds, and percentage of total germination). A Poisson estimation, using a “log” link function and setting the variance to “mean”, was used when the distribution of the response variable was Poisson like (i.e., number of capitula, total flower number, etc.) (Venables and Ripley 1998). The models were built with population density, aggregation index, population structure ratio, and plant diameter as fixed variables, and “population identity” as a random variable with “plant identity” nested in the population identity variable. Total number of capitula per plant was not included in the model because it was highly correlated with plant size. To test the interannual variability in the modelled variables, we also performed a GLMM with data from the three populations visited in 2002 and 2003. This variable was included as a fixed effect because of the low number of df (1). Because our data were unbalanced, we used Satterthwaite’s method

Table 2. Generalized linear mixed models (GLMM) for variables at plant level as dependent variables and plant and population-level variables as independent predictors.

Dependent variables	Independent variables					
	Population-level variables				Plant-level variables	
	Density	Pop. str.	Plant aggr.	Pop. iden. (R)	Size	Plant iden. (R)
Total capitula (<i>P</i>)	NS	NS	NS	NS	0.042***	
Total flower number (<i>P</i>)	NS	NS	NS	NS	0.045***	
Total viable seeds (<i>P</i>)	NS	NS	NS	NS	0.045***	
Total aborted seeds (<i>P</i>)	NS	NS	NS	NS	0.004***	
Seed set (<i>B</i>)	-0.022*	NS	0.8*	NS	NS	
Mean viable seeds per capitulum (<i>P</i>)	NS	NS	NS	NS	0.005***	
Germination percentage (<i>B</i>)	NS	NS	NS	NS	NS	
Seed mass (5 seeds per plant) (<i>P</i>)	NS	NS	0.3*	NS	0.004***	0.04***

Note: Population identity was introduced in the model as a random variable (*R*). Estimation coefficients and significance levels are represented. Effects of random factors were tested using Wald Z-statistic tests, and those of fixed factors were tested with *F* tests. Degrees of freedom for density, population structure, and plant aggregation varied between 3.59 and 4.35, whereas degrees of freedom for plant size ranged between 315 and 336 (Satterthwaite's method). *P*, Poisson-like error distribution; *B*, binomial error distribution; pop. str., population structure; plant aggr., plant aggregation index; pop. iden., population identity. *, statistical significance at $0.02 < p < 0.05$; ***, statistical significance at $p < 0.001$; NS, nonsignificant.

to determine the approximate denominator degrees of freedom for tests. Plants were nested within populations, and plants and populations were considered as random factors (variance components). Because of the complexity of the model structure and the relatively large number of potential explanatory variables, all possible combinations of interactions and polynomials could not be fitted, and the model was kept relatively simple considering main effects only. Effects of random factors were tested using Wald Z statistic tests, which are appropriate for large samples, and those of fixed factors were tested with *F* tests, which are preferable when the structure of the variance-covariance matrix depends on an unknown scale parameter. We also fitted an unconditional means model for each target variable to examine the variation associated with each hierarchical level (Littell et al. 1996). These models exclusively including random variables provided a base line against which we could compare a more complex model with fixed effects. Statistical analyses were performed using the SAS statistical package (SAS Institute Inc. 1996). A SAS macro program GLIMMIX (version 19 available on the World Wide Web at <http://support.sas.com/ctx/samples/index.jsp?sid=536&tab=downloads>) was used. The MIXED procedure implements a generalization of the standard linear model that allows for proper incorporation of random effects (Littell et al. 1996; SAS Institute Inc. 1996).

Results

Individuals of *C. hyssopifolia* showed high variability in total flower number, total viable seeds, and seed set per capitulum and per plant. In this study we considered 351 plants and 2591 capitula in 2002 and 150 plants and 1144 capitula in 2003 (Table 1). Mean seed set for all populations (0.33) was a typical value of self-incompatible hermaphrodite plants (Sutherland and Delph 1984). Mean germination percentage was 44%, and most remarkably, the larger seeds were 11-fold heavier than the smaller fully developed ones. The seven studied populations showed the following range of variability in the three population variables considered:

(a) plant density ranged from 0.20 plants·m⁻² to 0.75 plants·m⁻²; (b) aggregation level measured with the Morisita index varied from 0.97 to 1.43 (from almost uniform to highly clumped spatial structure); and (c) plant size structure ranged from 0.59 to 6.7 (from populations dominated by small-sized plants to populations dominated by large-sized individuals).

Aggregation index positively affected seed set, while population density exerted a negative effect on seed set, either measured at plant or at capitulum level (Tables 2 and 3; Fig. 1). The proportion of ovules that set seeds decreased in denser populations ($p = 0.04$) but significantly increased in clumped populations ($p = 0.016$). Plant size structure did not exert any effect on the plant reproduction variables measured.

Plant aggregation also exerted a marginally significant positive effect on seed mass ($p = 0.055$) (Table 2; Fig. 2). This suggests that plants growing in populations with clumped individuals developed heavier seeds than those growing in populations with uniformly distributed plants. Total germination percentage was not affected by any of the plant or population variables measured (Table 2).

Plant size significantly controlled most of the target variables at plant level (Table 2; Fig. 3). As expected, plant size was positively associated to the number of capitula, flowers, and viable, aborted, and parasitized seeds per plant. Seed mass was also significantly controlled by plant size with a positive coefficient. Similarly, plant size determined the total number of flowers and the amount of aborted seeds produced by each capitulum.

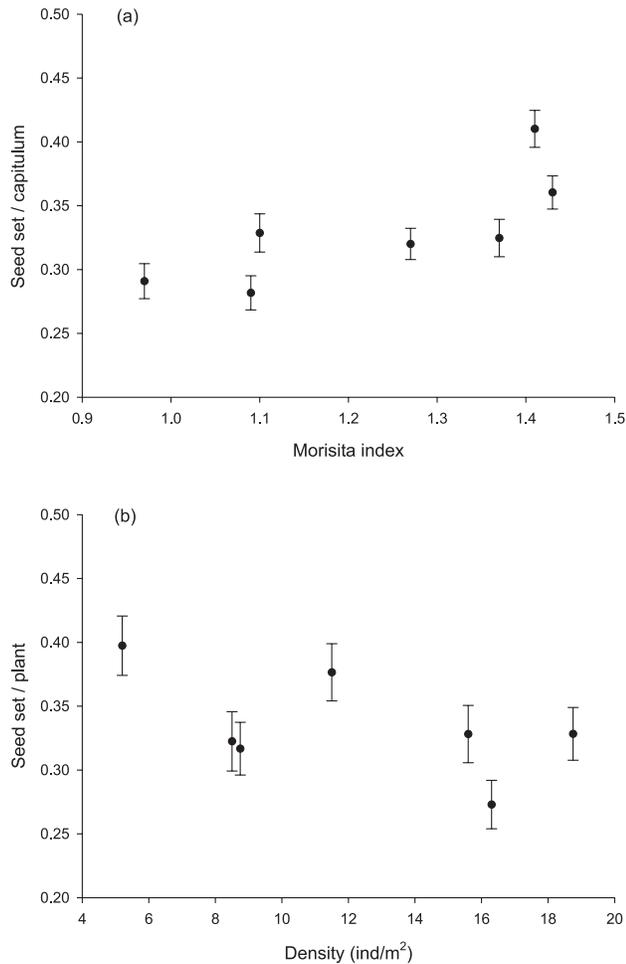
In the corresponding unconditional means models, hypothesis testing of variance estimates revealed that "plant (population)" or "plant identity" significantly differed from zero in those variables measured at the capitulum level (Table 3), but not in the case of population (Tables 2 and 3). This suggests the existence of other nonmeasured sources of variation such as genetic and (or) maternal effects at the plant level for most modelled variables.

Finally, no between-year effect was observed in this study

Table 3. Generalized linear mixed models (GLMM) for plant fitness variables at capitulum level as dependent variables and plant and population-level variables as independent variables.

Dependent variables	Independent variables					
	Population-level variables				Plant-level variables	
	Density	Pop. str.	Plant aggr.	Pop. iden. (<i>R</i>)	Size	Plant iden. (<i>R</i>)
Flower number (<i>P</i>)	NS	NS	NS	NS	0.0045***	0.03***
Viable seeds (<i>P</i>)	NS	NS	NS	NS	NS	0.14***
Aborted seeds (<i>P</i>)	NS	NS	NS	NS	0.0039*	0.09***
Seed set (<i>B</i>)	-0.025*	NS	0.9*	NS	NS	0.28***

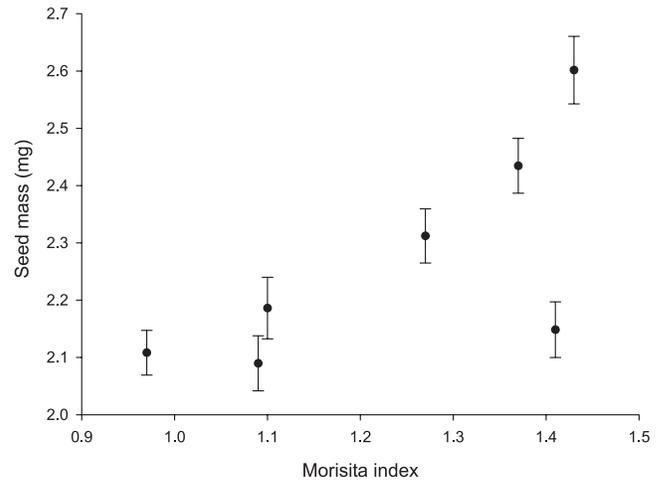
Note: Population and plant identity were introduced in the model as random variables (*R*). Estimation coefficients and significance levels are represented. Effects of random factors were tested using Wald Z-statistic tests, and those of fixed factors were tested with *F* tests. Degrees of freedom for structure and plant aggregation varied between 3.63 and 4.38, whereas degrees of freedom for plant size ranged between 320 and 346 (Satterthwaite's method). *P*, Poisson-like error distribution; *B*, binomial error distribution; pop. str., population structure; plant aggr., plant aggregation index; pop. iden., population identity. *, statistical significance at $0.01 < p < 0.05$; ***, statistical significance at $p < 0.001$; NS, nonsignificant.

Fig. 1. Mean seed set per capitulum \pm SE for the seven populations of *Centaurea hyssopifolia* identified by their Morisita indexes and mean seed set per plant \pm SE for the seven population density values in 2002 (350 plants and 2591 capitula; $n = 50$ per population).

for any variable ($p > 0.25$), thus, no change of the general trend happened between both study years (2002 and 2003).

Discussion

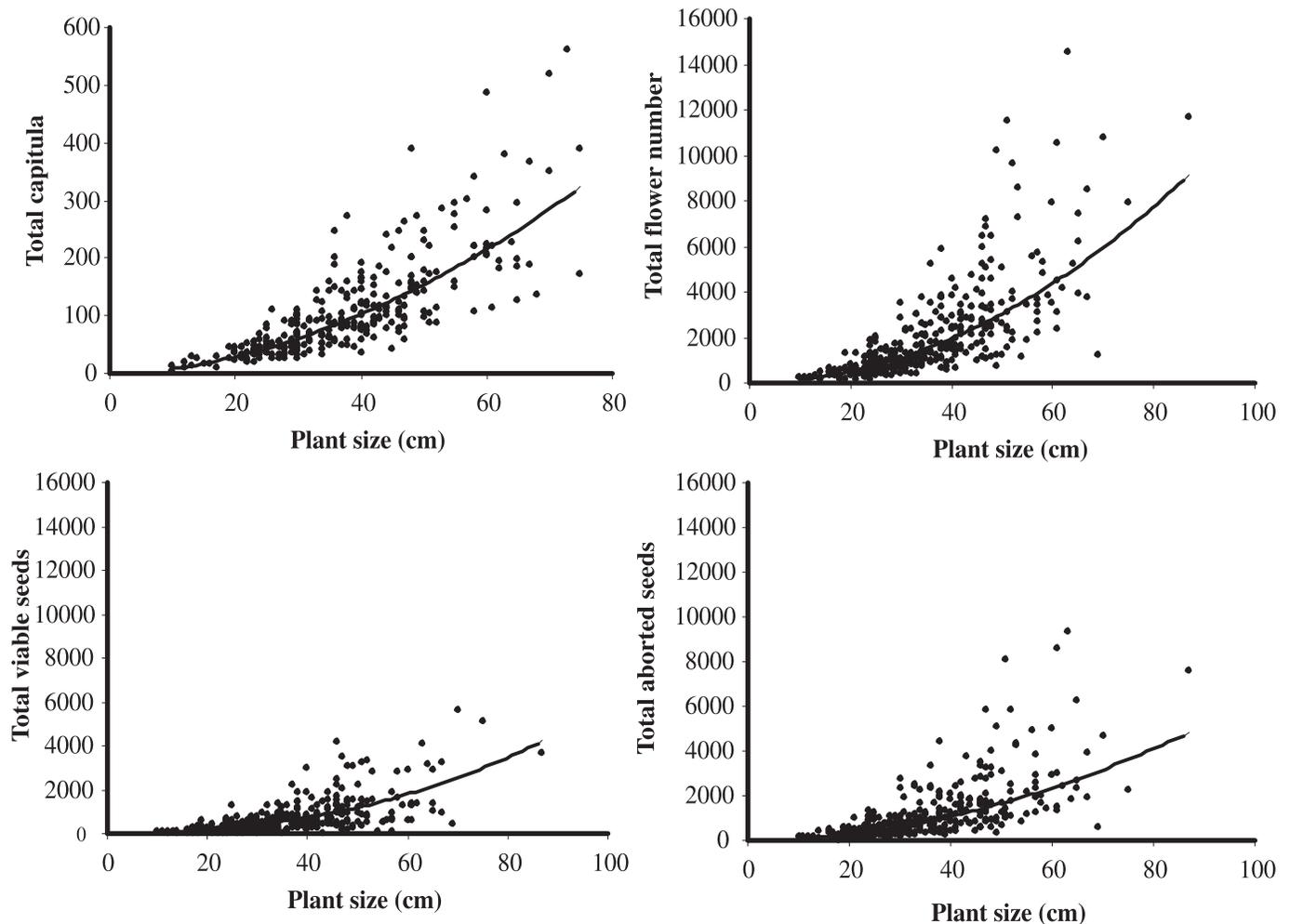
Our results confirm our hypothesis that population-level

Fig. 2. Mean seed mass \pm SE for the seven populations of *Centaurea hyssopifolia* identified by their Morisita indexes in 2002. Data are from 5 seeds per plant (350 plants and 2591 capitula; $n = 50$ per population).

characteristics affect reproductive success of a rare semiarid plant species. Some studies have demonstrated the effect of some landscape factors such as patch size and isolation degree on the reproductive success (Schulke and Waser 2001; Jacquemyn et al. 2002); however, in this study we went beyond these landscape predictors and showed that plant aggregation, plant density, and plant size structure exerted a significant effect on plant reproduction.

We found more aggregated plants to have greater seed set. A seed set increase in clumped populations together with a seed set decrease in sparse ones have been previously observed (Watanabe et al. 2003). The positive effect of the aggregation index at plant and capitulum level may be caused by the increase of pollination efficiency at those places where plants were aggregated. Sparse plants might receive more pollen from flowers in the same plant (geitonogamy), because pollinators tended to visit more flowers per plant before moving to the next plant (Charnov 1976; Pyke 1984; de Jong et al. 1993). In populations of sparse plants, pollination efficiency may decrease because generalist pollinators usually visit several species when travelling from one plant to another (Kunin 1993, 1997a), and consequently, the proba-

Fig. 3. Relationship between plant size and total capitula (a), total flower number (b), total viable seeds (c), and total aborted seeds per plant (d) of *Centaurea hyssopifolia*. The line represents the best adjusted equation for the data. Includes plants from both sampling years ($n = 500$ plants).



bility for the pollinator to transfer conspecific pollen decreases with the distance between conspecific plants (Kunin 1993, 1997a; Aizen and Feinsinger 1994). The delivery of heterospecific pollen can clog stigmas and consequently lower reproductive success (Petanidou et al. 1998).

Seed mass also increased in aggregated populations (Fig. 2). This result reinforces the hypothesis that populations with clumped plants favour several aspects of plant fitness. Spatial aggregation may favour mixed conspecific fertilization (Duncan et al. 2004), and consequently multiple paternities because of pollen grain competition, this is supposed to increase plant fitness of insect-pollinated self-incompatible plants (Marshall 1990, 1991). Higher competition among pollen grains may result in higher seed quality. Although clumped populations showed higher seed set and seed mass, we did not detect any effect on other overall reproductive variables measured at plant level such as total number of viable seeds (Tables 2 and 3).

In spite of plants from aggregated populations showing heavier seeds, we did not detect subsequent differences in germination percentage. The lack of differences in germination percentage may be due to two possible reasons. First, maternal effects usually determine germination response to

a large extent (Oostermeijer et al. 1994; Luzuriaga et al. 2006). Second, the favourable laboratory conditions for germination could have masked the differences among populations that would only be expressed under the more physiologically demanding field conditions (Ramsey and Vaughton 1998).

Simultaneous inclusion of plant density and plant aggregation in the fitness models led to a more accurate interpretation of both population attributes. We suggest that the apparent contradiction between both variables — the positive effect of plant aggregation and the negative effect of plant density on seed set — may be explained by the fact that each variable acts at different spatial scales. Since the aggregation index variable better explained nearness among plants, the GLMM modelling excluded the aggregation component out of the plant density variable, and consequently it should be interpreted without this component. The aggregation index acts at the neighbourhood scale (i.e., attractiveness), whereas density does at larger scales.

We propose that the negative effect of density may lie in the harder competition conditions created in denser populations, especially in the highly stressful environment inhabited by this species (Harper 1977; Antonovics and Levin

1980; Weiner 1982; Pacala and Silander 1990). In dense populations, competition for resources (most likely water in semi-arid environments) may be tougher than in sparse populations, and consequently the resource limitation suffered by plants in dense populations may affect their reproductive output.

We expected that population plant size structure would affect reproductive output, since the higher the frequency of large plants in the population, the more attractive plants would be for pollinators. The lack of population size structure effect on plant reproductive output in this study was probably related to the fact that *C. hyssopifolia* is a generalist-pollinated species (A.L. Luzuriaga, personal observation). The studied populations were immersed in a typical Mediterranean insect-pollinated shrub community and consequently, abundance and density of pollinators were independent of *C. hyssopifolia* population size structure (Kunin 1997b; Oostermeijer et al. 1998; Leimu and Syrjänen 2002).

Interestingly, in this study we did not detect any significant effect of population identity on reproduction and fitness (Tables 2 and 3). The effect of population was distributed among the structure attributes (density, aggregation index, and population size structure), and thus, there was no remaining variability to be explained by population identity, at least when population size was high enough to minimize the effect of any landscape variable. These results highlight the importance of variance partitioning to understand the mechanisms underlying interpopulation variability, beyond population identity itself. Our results showed that population-level processes are key factors that significantly contribute to plant reproductive success.

Plant size versus plant identity

As expected, plant size positively affected the mean number of viable seeds per capitulum at plant level (Samson and Werk 1986; Schmid and Weiner 1993; Pino et al. 2002; Sans et al. 2002), but we detected no significant effect of plant size on the number of viable seeds per capitulum. The reason of these apparently contradictory results between both levels of analyses (plant and capitulum) is explained in the light of the analyses done. In the plant-level analysis, we only considered the plant size variable, while in the capitulum-level analysis, the total effect was partialled out into two complementary sources of variation: plant size and plant identity. When taking together both variables, the effect of plant identity was removed, which otherwise would be included in the effect of plant size. Consequently, the positive effect of plant size on mean viable seed production per capitulum detected at plant level might be, at least partially, the masked effect of plant identity (i.e., genetic and maternal effects). The capitulum-level analysis showed that the higher production of flowers in larger plants was not reflected in more viable seeds, since the number of aborted seeds also increased (Table 3). We could infer that resources were distributed among a similar number of seeds rather than increasing the total number of viable seeds.

Although we expected that large individuals would receive more pollination services than small ones because of their higher attractiveness to pollinators, our results showed that plant size did not affect seed set, neither at plant nor at

capitulum level. These results emphasize the idea that pollination in *C. hyssopifolia* was independent of the attractiveness at the individual (plant size) or at the population (population size structure) level, probably because pollinators were not a limiting variable in this environment.

Plant identity was the main source of variation of plant reproductive output in our models (Table 3). Obviously, the large influence of plant size on reproduction may reflect that genotype characteristics together with phenotypic plasticity were the main factors controlling reproduction in *C. hyssopifolia*. In summary, differences in reproductive output at capitulum and plant levels did not only depend on plant characteristics (small-scale processes), but also on population spatial structure at least in large enough populations as considered in this study. Our results emphasize the importance of considering variables measured at different scales, such as plant and population-level variables to unveil the factors controlling the reproductive success (Kunin 1997b). Another relevant finding of this study is that the reproductive and fitness trends were similar in both study years.

Population-level variables seem useful to evaluate the effect of habitat fragmentation on plant reproduction. Since these variables are easily measurable and provide valuable information about habitat fragmentation effects on plant reproductive characters, we pose that predictions on reproduction in fragmented landscapes can be improved. This is especially relevant for the conservation management of rare plant species, and it would be useful to establish range values of population attributes in which the species may reproduce successfully.

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