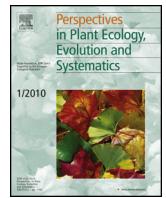




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Research article

## Does size matter? Ontogenetic responses of an Andean shrub to conspecific density-dependence



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### ABSTRACT

Dispersal, population spatial structure and intraspecific density-dependent processes are critical determinants of plant population dynamics. However, we lack empirical data on how the interplay between these factors changes across the plant species life cycle, particularly for long-lived plants. This is in great part due to the difficulty in implementing experimental approaches to discriminate the relative effects of these factors. Here, we studied the spatial pattern of adults and recruits of the Andean shrub *Croton wagneri* Müll. Arg. (Euphorbiaceae) and assessed the role of dispersal, population spatial structure and intraspecific interactions on individual plant performance. For this purpose, we developed a spatially explicit approach in which plant spatial position is combined with vegetative and reproductive attributes. The spatial pattern of *C. wagneri* showed small-scale aggregation. This is consistent with the species' short-distance seed dispersal. The spatial pattern of recruits was dependent on the spatial pattern of adults, signifying a high relevance of adult-recruit interactions in the spatial pattern creation. However, once established, recruits seemed to be subjected to self-thinning (*i.e.*, negative density dependence). Finally, a positive density-dependent effect of the conspecific neighbourhood was found on adult reproductive performance, while plant growth was affected by intraspecific competition. Overall, dispersal, population spatial structure and intra-specific interactions combine to influence vegetative and reproductive performance of the species. We also found that plant interaction at the intraspecific level can be a composite of facilitative and competitive effects that combine in complex ways to influence population spatial structure and individual plant performance. Furthermore, such effects did not always leave a spatial signature, which highlights the importance of complementing plant position with quantitative fitness attributes in spatial pattern analysis in plant ecology.

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### 1. Introduction

Seed dispersal provides the basic template on which environmental filters and biotic interactions act to determine recruitment. This leaves a signature in the spatial pattern of the species, both in the spatial seedling – adult association and in the clustering of seedlings (Antonovics and Levin, 1980; Seidler and Plotkin, 2006; Escribano-Avila et al., 2014; Lara-Romero et al., 2014). This signature can subsequently be modified by density dependent processes (Antonovics and Levin, 1980; Seidler and Plotkin, 2006; North and

Ovaskainen, 2007). Indeed, numbers and distances to neighbours can change substantially during ontogeny, especially if negative density dependence affects early life stages such as saplings or seedlings (Stoll and Prati, 2001; Miriti, 2006; Eränen and Kozlov, 2008). This has important ecological consequences because individual plants with different neighbourhoods experience different growth, survival, and reproductive conditions (Silander and Pacala, 1985; Stoll and Prati, 2001; Lara-Romero et al., 2016), which deeply influences population dynamics.

It is readily acknowledged that experimental manipulative approaches are required to provide deeper insight into the underlying mechanisms of conspecific and interspecific plant spatial patterns (McIntire and Fajardo, 2009). However, this approach is practically infeasible for studying long-lived adult plants (McIntire and Fajardo, 2009; Wiegand and Moloney, 2014), particularly when

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the goal of the study is to investigate the effect of plant – plant interactions on succession. State-of-the art techniques of spatial point pattern analysis can help identify possible mechanisms underlying observed plant spatial patterns (Wiegand and Moloney, 2014). These analyses are based on the fact that facilitation and competition are processes primarily occurring among neighbouring plants and therefore spatial locations of plants provide information for inferring the sign and strength of plant – plant interactions in field conditions. Regarding competition, regular spatial patterns of surviving individuals are usually interpreted as evidence for asymmetric competitive interactions and subsequent density-dependent mortality (Stoll and Bergius, 2005; De La Cruz et al., 2008). This process can also be detected via decline in aggregation with increasing size/age classes due to natural thinning (Getzin et al., 2008b; Chacón-Labela et al., 2014). Conversely, facilitative interactions among conspecifics are frequently considered a main driver of spatial aggregation patterns observed among plants at small spatial scales (Haase et al., 1996; Montesinos et al., 2007; Gimeno et al., 2012).

However, spatial pattern alone is not always sufficient in the detection of competition or facilitation, as plant patterns result from a diverse array of ecological factors that can be antagonistic or cooperative: seed dispersal (North and Ovaskainen, 2007; Jara-Guerrero et al., 2015), intraspecific and interspecific interactions (Lortie and Turkington, 2008; Espinosa et al., 2014; Lara-Romero et al., 2016), environmental heterogeneity (Getzin et al., 2008b; Lara-Romero et al., 2016), seed predation (Jansen et al., 2014), herbivory and disease (Comita et al., 2014). Indeed, plant – plant interactions may not be strong enough to cause significant spatial signal, but rather may affect many vegetative or reproductive variables (Silander and Pacala, 1985; Weiner, 1988; Jones and Comita, 2008). Furthermore, dense neighbourhoods can either result in competitive and facilitative processes (Weiner, 1988; Jones and Comita, 2008; McIntire and Fajardo, 2011). Hence, a more consistent approach is to complement analysis of plant spatial patterns with quantification of the effect of neighbourhood conditions on individual performance (i.e., growth, size or reproductive output), which can provide a measure of the sign and strength of plant – plant interactions (Getzin et al., 2008b; Gray and He, 2009; Lara-Romero et al., 2016).

Despite the importance of spatial structure in plant population dynamics, we still do not know to what extent aggregation and segregation in plant populations can be attributed to the existence of positive and negative interactions among their individuals. Furthermore, little is known about how the relevance of the different factors affecting spatial patterns changes across the plant species life cycle (but see Miriti, 2006; Eränen and Kozlov, 2008; McIntire and Fajardo, 2011). Here we developed an observational approach combining several spatial analyses to evaluate the relationship between plant population spatial patterns, plant demography and intraspecific interactions in the Andean shrub *Croton wagneri* Müll. Arg. (Euphorbiaceae). In this approach, the spatial position, growth, size and reproductive output of individual plants is considered to be a function of its neighbourhood conditions. We additionally assessed whether these relationships varied locally by comparing not only a gradient *sensu stricto*, but also two sites with contrasting elevations that are representative of mild and harsh conditions for the species.

*C. wagneri* comprises most of the biomass in the Ecuadorian Interandean Dry Scrub ecosystem, where it acts as a key ecosystem engineer by providing refuge and shelter for many other species (Espinosa et al., 2014). Therefore, its spatial structure governs the functioning of these communities. Previous studies found that *C. wagneri* populations have an aggregated spatial distribution and the intensity of the spatial aggregation varies along an elevation gradient in response to changing environmental conditions

(Espinosa et al., 2014; Ramón et al., 2016). The ballochorous dispersal system suggest that most seeds are dispersed at short distance (Jara-Guerrero et al., 2015). We therefore expected strong aggregation of adults and recruits within populations and pronounced spatial association between recruits and adults (Seidler and Plotkin, 2006; Lara-Romero et al., 2014; Jara-Guerrero et al., 2015). We additionally expected that the aggregated template generated by seed dispersal and recruitment would be subsequently modulated by competitive plant – plant interactions (Seidler and Plotkin, 2006; North and Ovaskainen, 2007). All these elements make *C. wagneri* a suitable species in which to assess the relationship between plant spatial patterns, demography and intraspecific interactions. We hypothesized that (1) the combination of limited seed dispersal and facilitative effects of adults on recruits would lead to clumps of recruits spatially aggregated around adult plants; (2) conspecific negative density-dependence would cause the spatial pattern of *C. wagneri* to become more regular throughout the ontogeny; (3) a significant negative spatial association between plants of different sizes would appear if competition affected growth, and (4) a negative density-dependent effect of the conspecific neighbourhood on reproductive performance if intra-specific competition affected reproductive allocation.

## 2. Material and methods

### 2.1. Study site and species

The study was conducted in one of the best-preserved Interandean dry montane scrub ecosystems of Southern Ecuador (Valley of Catamayo, Loja province, 03°58'29''S, 01°25'22''W), which covers an altitudinal gradient from 1400 to 1700 m.a.s.l. The annual average temperature and rainfall range from 33.8°C and 423 mm respectively in the lower zone to 27.4°C and 562 mm in the upper zone. The rainy season occurs between February and April and the dry season between May and January (Espinosa et al., 2014). Vegetation forms conspicuous perennial patches dominated by *Croton wagneri*, which are interspersed with bare soil areas (occupied by annual plants during the rainy season). *C. wagneri* is an evergreen shrub up to 2-m high with open, actinomorphic and monoecious flowers. The species is available to generalist flower-visiting insects such as small bees and flies (Armbruster et al., 1999). Its seeds have an average mass of 0.75 mg and a diameter of between 2 and 4.5 mm. The species is essentially ballochorous (seeds are forcefully ejected by dehiscence) and most seeds are thought to be dispersed only a few meters (Jara-Guerrero et al., 2015).

### 2.2. Data collection

We established two study plots (with dimensions of ca. 50 m x 70 m) at both “low-edge” (1400 m.a.s.l.) and “high-edge” (1700 m.a.s.l.) elevations. These are local extremes which represent harsh and mild conditions for the species, respectively (Espinosa et al., 2014; Ramón et al., 2016). We mapped all *C. wagneri* adult plants and recruits in each study plot using a Leica total station, model TS02-5power with a precision of <5 cm. Maps of the spatial distribution of *C. wagneri* in the plots are provided in Fig. S1 in the Appendix A. Recruits were defined as the plant individuals with less than 70 cm<sup>2</sup> in crown area not showing flowering or fructification signs over the last two years. Our definition of recruits includes individuals that germinated and survived at least their second full summer. With this framework we included all critical stages of effective dispersal: seed dispersal, seed germination and seedling survival.

### 2.3. Spatial summary statistics

We employed the univariate pair correlation function  $g(r)$  to characterize the spatial distribution of plants in the study plots. It is related to Ripley's  $K(r)$  in the form  $g(r) = K'(r)/(2\pi r)$  (Wiegand and Moloney, 2014). It gives the expected number of points in a ring at distance  $r$  from an arbitrary point, divided by the intensity of the pattern in the study area (i.e., plant density). Bivariate extension of the pair correlation function  $g_{ar}(r)$  and  $g_{ra}(r)$ , comprising type "a" and type "r" points (where type "a" represents adult plants and type "r" recruits) are defined as  $K'_{ar}(r)/(2\pi r)$  and  $K'_{ra}(r)/(2\pi r)$ , respectively (Wiegand and Moloney, 2014). Function  $g_{ar}(r)$  estimates the number of recruits found at a distance  $r$  around an arbitrary adult, and  $g_{ra}(r)$  the number of adults around an arbitrary recruit. We computed  $g(r)$  function up to 8 m at 0.05 m intervals, using isotropic edge correction.

### 2.4. Characterization of the spatial pattern of recruits and adult plants

To characterize the magnitude of aggregation of recruits and adults in each plot, we fitted a Poisson cluster process (PCP) using the minimum contrast method (Diggle, 2014). According to this method, a summary function, such as  $K(r)$  or  $g(r)$  is computed from the data point pattern. The theoretical expected value of this summary function is derived as an algebraic expression involving the parameters of the model. Then the model is optimized to find the best fit between the theoretical and empirical curves (Baddeley and Turner, 2005). A PCP describes the formation of a pattern as a two-step process. First, a Poisson pattern of "parent" points is generated with intensity  $\rho$ . Next, each parent point produces "offspring," where the number of offspring per parent follows a Poisson distribution, and their locations are independent and normally distributed around the parent points (i.e., isotropically), with a mean of zero and standard deviation of  $\sigma$ . The aim of fitting the PCP is to estimate  $\sigma$  and  $\rho$ , which measure the magnitude of aggregation, and to identify possible differences among populations and stage classes (Jacquemyn et al., 2007; Lara-Romero et al., 2016). A previous study in the same species and study site found that the species exhibited clear aggregated patterns compatible with PCPs (Ramón et al., 2016).

### 2.5. Differences in aggregation and spatial relationships between recruits and adult plants

We used the null model of random labelling within a case-control design to test whether clustering strength varies between recruits and adults and to explore the spatial relationship between stage classes. The random labelling null model assumes that cases are a random subset of the population group composed of cases and controls (De La Cruz et al., 2008; Getzin et al., 2008b). We used recruits as "cases" and compared them to the pattern of adults (i.e., controls). Random labelling implies that  $g$ -functions are invariant, and therefore, we expected that  $g_{rr}(r) = g_{aa}(r) = g_{ar}(r)$ . Departure from random labelling indicates that a non-random process determines whether a point was case or control.

#### 2.5.1. Recruit-adult relationships

To test whether the pattern of recruits is significantly associated with the pattern of adults, we employed the differences  $g_{aa}(r) - g_{ar}(r)$ , and  $g_{rr}(r) - g_{ra}(r)$ , which have an expected value of 0 under random labelling. Hence, if  $g_{aa}(r) - g_{ar}(r) = 0$ , adults would be surrounded by the same density of adults and recruits, indicating that adult and seedlings exploit the available habitat in the same way. Negative deviations at short scales would indicate the attraction of recruits to adults (i.e., recruitment near adults). Con-

versely, positive departures from the null model would indicate segregation between adults and seedlings (Getzin et al., 2008b; Chacón-Labelle et al., 2014). A value of  $g_{rr}(r) - g_{ra}(r) > 0$ , would indicate the existence of an additional clustering mechanism within recruits, independent of the pattern of adults (Getzin et al., 2008b).

#### 2.5.2. Differences in the strength of clustering from seedlings to adults

We employed the difference of functions,  $g_{aa}(r) - g_{rr}(r)$  to detect differences in the strength of clustering from seedlings to adults. This function has an expected value of 0 for all  $r$  distances if the pattern of adults remains as clustered as the pattern of recruits. Significant negative differences indicate that recruits are more clustered than adults, suggesting the existence of a thinning process (Getzin et al., 2008b; Lara-Romero et al., 2016).

### 2.6. Scale-dependent effects on plant growth

Distance-dependent effects on plant growth are easily recognizable via the spatial range and the strength of spatial correlation in plant size (Getzin et al., 2008a). To test the existence of distance-dependent size correlation we computed the mark correlation function  $k_{mm}(r)$  (Getzin et al., 2008a). We used crown areas as marks because crown extent is more sensitive than stem diameters to changes of neighbourhood density (Getzin et al., 2008a; Gray and He, 2009). The similarity or dissimilarity between the marks (i.e., crown area) of two trees at a distance  $r$  apart is quantified by the equation  $f(c_1, c_2) = c_1 \times c_2$ , where  $c_1$  and  $c_2$  are crown areas of two neighbouring trees.  $k_{mm}(r)$  is the normalized mean value of  $f(c_1, c_2)$  for all marks at distance  $r$ . Marks are considered independent if  $k_{mm}(r) = 1$ , and positively or negatively correlated at distance  $r$  if  $k_{mm}(r) > 1$   $k_{mm}(r) < 1$ , respectively.

### 2.7. Model evaluation

To evaluate the significance of the implemented spatial null models, 95% simulation envelopes were computed from 399 Monte Carlo simulations of each null model. The simulation envelopes cannot be interpreted as confidence intervals because the null hypothesis is tested simultaneously at many scales, which may inflate Type I error (Loosmore and Ford, 2006). We therefore evaluated the overall fit of each model with a goodness-of-fit (GOF) test using the  $u$  statistic (Loosmore and Ford, 2006; Diggle, 2014),

$$u = \int_{r=0}^{r=8} \{ \hat{g}(r) - \bar{g}(r) \}^2 dr$$

where  $\hat{g}(r)$  is the empirical estimation of the pair correlation function,  $\bar{g}(r)$  the mean of the pair correlation functions of 399 patterns simulated according to the evaluated null model. Therefore,  $u$  represents the total squared deviation between the empirical pattern and the theoretical result across the distances of interest. If  $u$  of the observed pattern is larger than those of 380 simulations (of the 399 total simulations), the null hypothesis is rejected at on a 5% level. We performed spatial analyses using the R packages "spatstat" v 1.42-2 (Baddeley and Turner, 2005) and "ecsp" v 2 (De La Cruz et al., 2008).

### 2.8. Density dependent effects on reproductive performance

In order to seek additional evidence of plant – plant interaction between adult plants, we fitted a linear model to test if the conspecific density around each adult affects individual reproductive performance. We fitted linear models for flowers per flowerhead per plant (hereafter flowers per flowerhead), seed dry weight and seed number per fruit per plant (hereafter seeds per fruit). To fit

models for flowers per flowerhead we randomly chose 270 individuals per site ( $n = 540$ ) and estimated the mean number of flowers produced in five flowerheads per plant. To fit models for seed dry weight and seed per fruit we randomly selected 35 healthy individuals at each site ( $n = 70$ ). We dissected four fruits per individual, counted the total number of seeds per fruits and obtained the mean value for each individual. To equalize sample sizes between individual we randomly selected four seeds per individual to estimate mean seed dry weight of each plant. The models included crown area of each adult plant, intensity of conspecifics around each adult, position in the elevational range (low and high) and their interaction as predictor variables. Intensity around each adult plant (hereafter neighbourhood density) was estimated using a Gaussian kernel with a standard deviation equal to the maximum distance to a nearest-neighbour in each study population (Diggle, 2014; Lara-Romero et al., 2016).

We assumed Gaussian error for all the models except for the model fitted for flowers per flowerhead, for which we assumed a Poisson error and a log-link function. Additionally, seed per fruit variable were ln-transformed to reach normality. QQ-plots and predicted vs. residuals plot were used to verify assumptions of the models (Zuur et al., 2009). Models were fitted with the R packages “stats” (R Core Team, 2015). Akaike information criterion (AIC) was used to evaluate full models and reduced models fitted for all variable combinations. This approach selects the “best” model (i.e., the model with the smallest AIC) and ranks the remaining models based on their AIC value. Models with  $\Delta\text{AIC} > 2$  relative to the best model were discarded, as they have less statistical support (Burnham and Anderson, 2002). In cases where a set of models (model confidence set) instead of a best model was obtained, we used a model averaging approach for multimodel inference. Model averaging is based

in accounting for Akaike weights ( $w_+$ ), the relative importance of variables ( $w_i$ ) and the averaged parameter estimates obtained from the models in the confidence set (Burnham and Anderson, 2002).

### 3. Results

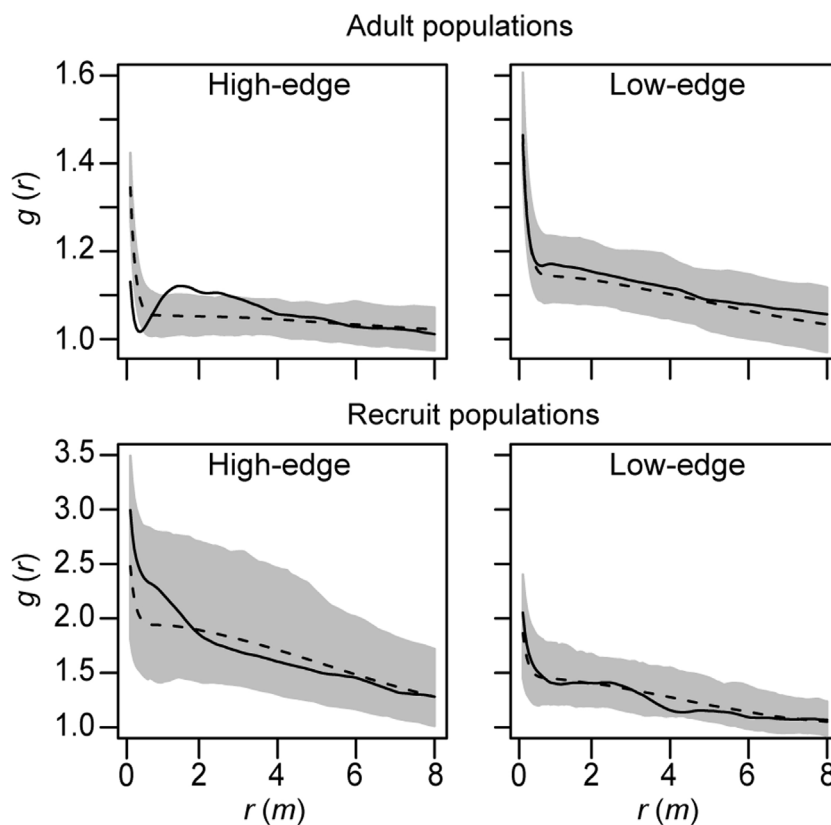
#### 3.1. Spatial pattern of recruits and adult plants

We mapped a total of 384 recruits and 2483 adults in the low-edge plot and 561 recruits and 2021 adults in the high-edge plot. The pattern of *C. wagneri* adults in the high-edge plot was slightly less aggregated at short-scales than expected under a Poisson cluster process (GOF test:  $p$ -value  $> 0.05$ ; Fig. 1). All the other adult and recruit populations were adequately described by a Poisson cluster process (GOF test: all  $p$ -values  $> 0.05$ ; Fig. 1).

The Poisson Cluster process describes a patchy distribution in which plant individuals are aggregated in clumps (clusters). Number of clusters ( $A\rho$ ) estimated was 20 and 75 for recruits and 268 and 153 for adults at high- and low-edge plots, respectively. According to mean cluster size (i.e.,  $r_c \approx 2\sigma$ ; Jacquemyn et al., 2007), the process was more clustered for recruits (10.77 and 7.93 m for high- and low-edge plots, respectively) than for adult plants (12.17 and 9.8 m for high- and low-edge plots, respectively). On a similar vein, the process was more clustered in low-edge plot than in high-edge plot.

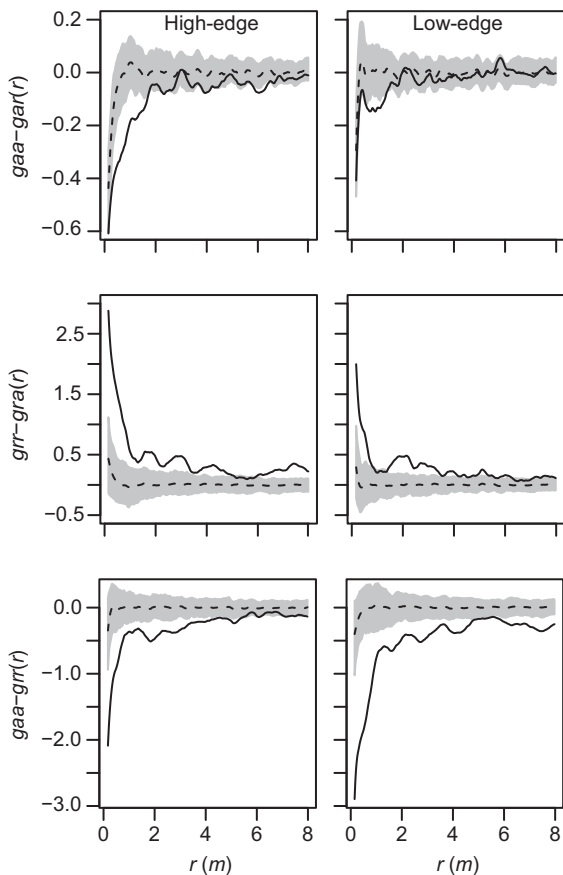
#### 3.2. Differences in aggregation and spatial relationship between recruits and adult plants

The empirical  $g_{aa}(r) - g_{ar}(r)$  difference negatively departed from the random labelling null model at short scales in all populations



**Fig. 1.** Spatial distribution of adults and recruits in *C. wagneri* study plots. Patterns of adults (top) and recruits (bottom) of *C. wagneri* are contrasted with the null model of Poisson cluster (PC) process using the  $g(r)$  function. The 95% simulation envelopes (grey area) were constructed using 399 simulations of the PC processes. The solid and dashed lines denote the observed pattern and the expectation of the null model, respectively. Note that y axes show different scales for clarity.





**Fig. 2.** Differences in the strength of clustering from seedlings to adults and analysis of the recruit-adult relationship. The figure shows differences between pair correlation functions  $g(r)$  using a null model of random labelling, where subscript 'a' and 'r' indicate adults and recruits of *C. wagneri*, respectively. The 95% simulation envelopes (grey area) were constructed using 399 simulations of the random labelling model. The solid and dashed lines denote the observed pattern and the expectation of the null model, respectively.

(GOF test: all  $p$ -value  $< 0.05$ ; Fig. 2), indicating recruitment near conspecific adults. On the other hand, the difference  $g_{rr}(r) - g_{ra}(r)$  revealed strong positive departures from random labelling at small

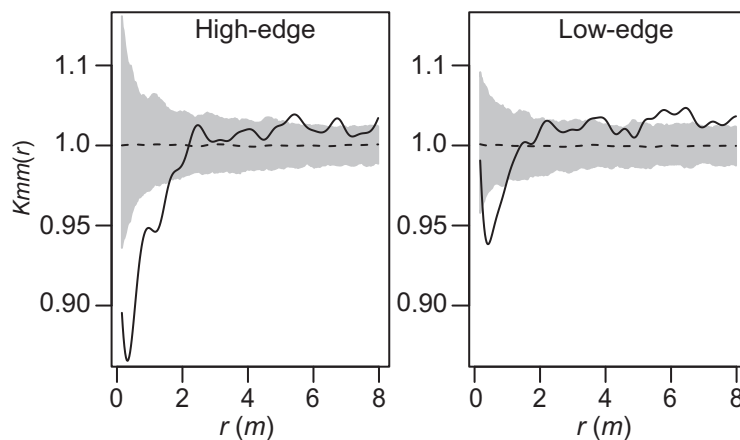
scales (GOF test: all  $p$ -value  $< 0.05$ ; Fig. 2), indicating an additional aggregation mechanism of recruits independent of the adult pattern. Finally, the difference  $g_{aa}(r) - g_{rr}(r)$  showed significant negative departures from the null model of random labelling (GOF test: all  $p$ -values  $< 0.05$ ; Fig. 2) in all populations, showing that clustering strength systematically declined from recruits to adults. This result is in agreement with estimated parameters for Poisson cluster process fitted for recruits and adult plants (see above) and may suggest the existence of a thinning process due to seedling mortality in the transition to adults.

### 3.3. Density-dependent effects on plant growth and vegetative and reproductive performance

Marked correlation function  $k_{mm}(r)$  applied to crown area indicated the existence of a significant negative correlation between marks as  $k_{mm}(r) < 1$  at small spatial scales in both plots suggesting small plants are associated by large neighbouring plants (GOF test: all  $p$ -value  $< 0.05$ ; Fig. 3). This suggests that intraspecific competition or other negative plant – plant interactions negatively affected plant growth in these plots.

A generalized linear model was selected according to AIC criterion for number of flowers per flowerhead (*i.e.*,  $\Delta AIC > 2$  with the rest of candidate models, Table 1). This model included crown area, neighbourhood density, position in the elevational range and the interaction between neighbourhood and crown area as predictors (Table 1). According to the coefficients of the model *C. wagneri* flower production was positively affected by conspecific neighbourhood density and plant size (Table 2). Nevertheless, the effect of neighbourhood density on reproductive performance was conditioned by focal plant size, with a more pronounced effect on small and medium-sized plants than on large plants; *i.e.*, the flowering output of larger plants was less affected by neighbourhood density than that of smaller plants (Fig. 4). Additionally, the production of flowers was lower at the low-edge populations than at the high-edge populations (Table 2 and Fig. 4).

Two linear models were selected according to AIC criterion for seed dry weight. These models included crown area and elevation as predictors (Table 1), which indicates that neighbourhood density did not affect seed weight of the study plants. Seeds from large plants and low-edge population were heavier than those of small plants located at high-edge population (Table 2). Regarding number of seeds per fruit, the highest ranked linear models

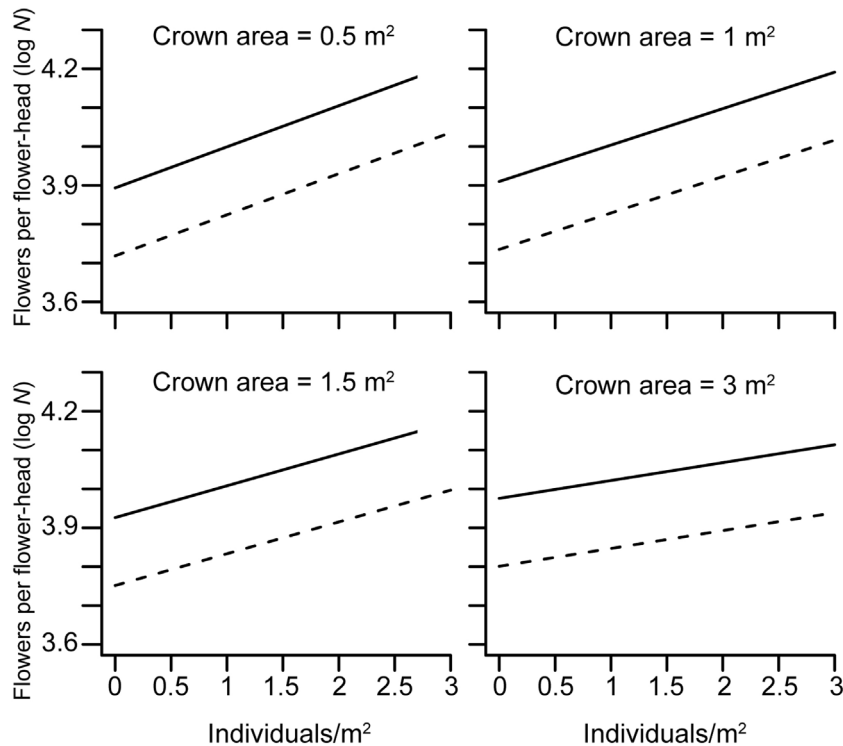


**Fig. 3.** Density-dependent effects on plant growth. The figure shows the marked correlation function  $k_{mm}(r)$  for crown area for adults of *C. wagneri*. The 95% simulation envelopes (grey area) were constructed using 399 simulations of the random marking null model. The solid and dashed lines denote the observed pattern and the expectation of the null model, respectively. Observed patterns that fall below the simulation envelopes indicate a negative correlation between trees of different marks (*i.e.*, crown area) at distance  $r$ .

**Table 1**  
Set of best-ranked linear models (i.e., models with  $\Delta\text{AIC} < 2$ ) fitted for vegetative and reproductive performance of *C. wagneri*.

Crown area	Neighbourhood	Elevation	Ngh:elev	Ngh:crown	log(L)	AICc	$\Delta\text{AICc}$	Akaike weight ( $w_i$ )	$R^2$
a) Number of flowers per flower heads									
X	X	X	–	X	–2405.28	4820.7	–	1	0.38
b) Seed dry weight									
X	–	–	–	–	22.54	–38.72	0	0.66	0.10
X	–	X	–	–	23.0	–37.38	1.34	0.34	0.11
c) Number of seeds per fruit									
X	X	–	–	–	–69.38	147.4	0	0.71	0.11
X	X	X	X	–	–67.9	149.1	1.7	0.29	0.15

Ngh:elev, interaction neighbourhood:elevation; Ngh:crown, interaction neighbourhood:crown-area; Log(L), maximised log-likelihood function;  $\Delta\text{AICc}$ , AICc differences;  $w_i$ , Akaike weights; na, not available.

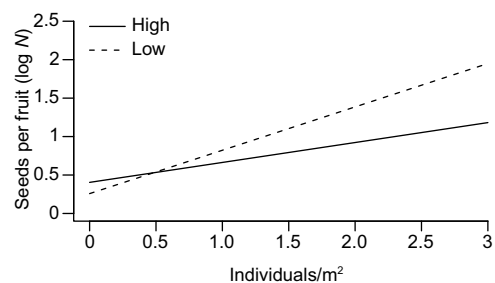


**Fig. 4.** Density dependent effects on flower production. LMs predictions (i.e. fitted models) for effects of neighbourhood conspecific density on *C. wagneri* flower production. Lines represent focal plants from different elevations. Panels represent focal plants of different crown area.

**Table 2**  
Relative importance ( $w_+$ ) and model-averaged estimates for variables selected using AICc-based model selection.

Effect	Estimate	SE	$w_+$
a) Number of flowers per flower heads			
Intercept	3.877	0.031	–
Crown area	0.033	0.007	1
Neighbourhood	0.118	0.035	1
Elevation (Low edge)	–0.175	0.012	1
Ngh:crown	–0.024	0.010	1
b) Seed dry weight			
Intercept	0.672	0.045	–
Crown area	0.029	0.011	1
Elevation (Low edge)	0.014	0.031	0.34
c) Number of seeds per fruit			
Intercept	0.268	0.189	–
Crown area	0.039	0.019	1
Neighbourhood	0.340	0.192	1
Elevation (Low edge)	–0.108	0.217	0.29
Ngh:elev (Low edge)	0.148	0.282	0.29

SE, standard error; Ngh:elev, neighbourhood density  $\times$  position in the elevational range interaction;  $w_+$ , relative importance estimates for variables. Intercept is the average of the dependent variables for the high-edge population when crown area and neighbourhood are zero.



**Fig. 5.** Density dependent effects on seed production. LMs predictions (i.e. fitted models) for effects of neighbourhood conspecific density on *C. wagneri* seed production. Lines represent focal plants from different elevations.

included crown area, neighbourhood density and position in the elevational range and their interaction as predictors (Table 1). According to these models, number of seed per fruit was positively affected by conspecific neighbourhood density and by individual plant crown area (Table 2 and Fig. 5). The significant neighbourhood density  $\times$  elevation interaction was due to a higher rate of increase of seed production with neighbourhood density in the low-

edge population compared to the high-edge population (Table 2 and Fig. 5).

#### 4. Discussion

Our results showed that dispersal, population spatial patterns and intra-specific interactions combine to influence vegetative and reproductive performance of *Croton wagneri* growing on Andean scrublands of Ecuador. Results were similar for both study sites with contrasting elevations, suggesting that environmental variation associated with elevational range may not be a major determinant of the spatial patterns of the species. A key result was the wide range of density-dependent responses of *C. wagneri* throughout the ontogeny. Furthermore, such responses did not always cause significant spatial signal, highlighting the importance of complement plant position by quantitative fitness attributes in spatial pattern analysis in plant ecology.

Short-distance dispersal by ballochorous dispersal syndrome may underlie aggregated establishment in *C. wagneri* populations, where seeds are thought to be dispersed only a few meters (Jara-Guerrero et al., 2015). However, seed recruitment not only depends on primary seed dispersal, but also on multiple biotic and abiotic factors controlling seed germination and further seedling establishment (Antonovics and Levin, 1980; Seidler and Plotkin, 2006; Lara-Romero et al., 2014). In this vein, direct facilitation of recruit establishment by adult *C. wagneri* plants might play a role in the observed pattern. This is supported by a companion study (Vélez-Mora, Quintana-Ascencio & Espinosa, Unpublished results) in which we have found that probabilities of *C. wagneri* seedling survival decrease with increasing distance from mother plants. Adult plants would facilitate establishment by modifying the microhabitat underneath them and alleviating abiotic stress (Antonovics and Levin, 1980; Fajardo and McIntire, 2011). This finding is consistent with the fact that the spatial pattern of recruits was dependent on the spatial pattern of adults. This is usually regarded as spatial signal of intraspecific adult-recruit facilitation (Getzin et al., 2008b; Chacón-Labela et al., 2014). Spatial heterogeneity of resource availability may also have led to clumping (i.e., Getzin et al., 2008b; Lara-Romero et al., 2016). However, this does not seem to be the case, as the spatial pattern of recruits and adults in each plot were well explained by a null model that assumed homogenous spatial distribution (i.e. Poisson cluster process). This is consistent with results reported by a related study performed in the same region (Ramón et al., 2016).

Recruits also showed some additional aggregation independent of adults that may be promoted by the clumping patterns generated by dispersal and the scarcity distribution of suitable sites for germination and establishment (i.e. safe sites) (Antonovics and Levin, 1980; Stoll and Prati, 2001). However, this result also matches a positive density dependent germination pattern (Antonovics and Levin, 1980). This may stem from biotic factors, such as intraspecific same-cohort facilitation, i.e. where higher conspecific seed or seedling density promotes seedling emergence and survival (Antonovics and Levin, 1980; Fajardo and McIntire, 2011; McIntire and Fajardo, 2011). Additional experimental work is required to provide deeper insight into the underlying mechanisms driving additional clustering of *C. wagneri* recruits (Stoll and Prati, 2001; Fajardo and McIntire, 2011; McIntire and Fajardo, 2011).

Initial recruitment was probably followed by a subsequent process of seedling mortality in agreement with the observed decline in clustering from recruits to adults (Antonovics and Levin, 1980; Getzin et al., 2008b). Intraspecific competition was also supported by the fact that small plants were associated with large neighbouring plants, indicating growth reduction under competition at small scales (Getzin et al., 2008a; Gray and He, 2009). This is com-

monly found in plant species with clumped seedling establishment (Antonovics and Levin, 1980; Stoll and Prati, 2001), where competition for space arises among neighbouring recruits, resulting most often in thinning to a single adult plant or to a few dominant and suppressed adult plants (Antonovics and Levin, 1980; De La Cruz et al., 2008). However, this pattern may also be explained by other biotic factors that depend on the neighbourhood of plants, such as herbivory, disease or mortality due to specialised natural enemies (Lortie and Turkington, 2008; Comita et al., 2014; Jansen et al., 2014;). Additional experimental work is needed to clarify which of the mechanisms is more relevant in the thinning of *C. wagneri* recruits. Despite the thinning process, the aggregated spatial structure induced by short-distance dispersal and subsequent seedling recruitment persisted within the adult class. This pattern of weak self-thinning that maintained initial aggregation has been previously reported in some other studies (e.g., Fajardo and McIntire, 2010; Lara-Romero et al., 2016). What is striking is the positive density-dependent effect of conspecific neighbourhood on reproductive performance. This result underlines that the balance between positive and negative density-dependence in our populations is complex, and most likely, acting in concert (Eränen and Kozlov, 2008; Goldenheim et al., 2008; Leger and Espeland, 2010; Fajardo and McIntire, 2011).

Growing evidence shows that inter- and intra-specific interactions can vary in strength or even switch direction depending on plant developmental status and size (e.g., Stoll and Prati, 2001; Miriti, 2006; Eränen and Kozlov, 2008; Lara-Romero et al., 2016). Accordingly, a set of studies have reported intraspecific, same-cohort plant facilitation (Harley and Bertness, 1996; Chu et al., 2008; Goldenheim et al., 2008; Leger and Espeland, 2010; Fajardo and McIntire, 2011). However, positive density dependence is still largely ignored in empirical studies of population dynamics, hampering research in this field (Bruno et al., 2003; Fajardo and McIntire, 2011). As a result, the circumstances under which neighbourhood interactions between conspecifics switch from negative to positive interactions are poorly understood (Bruno et al., 2003; Fajardo and McIntire, 2011; McIntire and Fajardo, 2011). The above-mentioned examples and other studies however, agree on the importance of intra-specific facilitation in high stressful environments, as in the case of the Ecuadorian Dry Mountain Scrub ecosystem dominated by *C. wagneri* (Espinosa et al., 2014).

The precise mechanisms driving observed density dependent effects at adult stage were not tested in the current study, yet the results provide possible explanations. The number of flowers per flower head was positively affected by conspecific neighbour density. Interestingly, the positive effect of conspecific neighbours on flower production decreased with focal crown area. Thus, smaller plants benefited more from neighbourhood density than larger plants, suggesting that reproductive allocation was affected by intraspecific competition for limited resources (Antonovics and Levin, 1980; Silander and Pacala, 1985; Lara-Romero et al., 2016). This is consistent with the high degree of abiotic stress and the harsh environmental conditions that prevail in the study site (Espinosa et al., 2014), tilting the balance in favour of facilitation. Factors responsible for this benefit may involve the amelioration of harsh environmental characteristics, the alteration of substrate characteristic or the introduction of beneficial organisms such as soil microbes or mycorrhizae (Antonovics and Levin, 1980; Bruno et al., 2003). The number of seed per fruit was also positively affected by conspecific neighbourhood density. Apart from the above mentioned mechanism, this may be due to the response of pollinators to flower density and dispersion (Sih and Baltus, 1987). Small clumps of animal pollinated plants are likely to be less attractive to pollinators than large clumps (Sih and Baltus, 1987; Ågren, 1996). As a consequence, pollinator visitation may be lower, and the degree to which seed production is limited by

pollinators may be higher, in smaller than in larger clumps (Sih and Baltus, 1987; Ågren, 1996). Our approach, however, cannot dismiss other factors that produce a density related reproductive response. Lower seed set and flower production by more isolated individuals might reflect lower reproductive capacity triggered by less favorable micro-environments or higher levels of interspecific competition (Albert et al., 2001). Hence, further research is needed to evaluate interplay between intraspecific aggregation, micro-environmental heterogeneity and interspecific competition and their effect on reproductive performance of the species.

## 5. Conclusions

This study shows that dispersal and recruitment is a determinant for the spatial pattern of *C. wagneri* growing on Andean scrublands of Ecuador. It also supports that plant interaction at the intraspecific level can be a composite of positive and negative density dependent processes that combine in complex ways to influence population spatial structure and individual plant performance of the species. Our results adds to a growing body of evidence highlighting the important role of positive intra-specific interactions in natural populations. The observed range of density-dependent responses of *C. wagneri*, and the finding that such responses may not cause significant spatial signal, point to the importance of measuring fitness parameters in point pattern based studies of plant – plant interactions. The knowledge gained through the study of this species could be extended to other dominant species with limited dispersal growing in environments with a high degree of abiotic stress, contributing to a better ecological understanding of Interandean dry montane scrub ecosystems. The spatial analyses applied in this study provide a useful approach to complement spatial point pattern analysis by quantifying the effect of neighbourhood conditions on individual performance. Further experimental manipulative studies are required to provide deeper insight into the balance between positive and negative density-dependence in our populations.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ppees.2017.02.002>.

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