Unraveling conflicting density- and distance-dependent effects on plant reproduction using a spatially-explicit approach

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Running title: Conflicting density- and distance-dependent effects
Summary

1. Density- and distance-dependent (DDD) mechanisms are important determinants of plant reproductive success (PRS). Different components of sequential PRS can operate either in the same or in different directions and thus reinforce or neutralize each other, and they may also operate at different spatial scales. Thus, spatially-explicit approaches are needed to detect such complex DDD effects across multiple PRS components and spatial scales.

2. To reveal DDD effects of different components of early PRS of the Iberian pear (*Pyrus bourgaeana*) sampled over three consecutive years, we used marked point pattern analysis. Our special interest is to identify conflicting processes that regulate populations at different spatial scales, e.g. whether DDD on fruit initiation and on fruit development acted in opposite directions. To evaluate the significance of observed mark correlation functions based on empirical data (e.g. fruiting success) we compared them to expectations given by spatially-explicit null models.

3. Diverse DDD processes affected several aspects of PRS in a variable extent over the three seasons. First, early fruit set was higher for individuals with more neighbors at small distances (i.e. up to 40m). However, late *P. bourgaeana* fruit set decreased with increasing number of nearby neighbors, but these effects canceled for overall fruit set that did not show DDD effects. Second, the absolute number of fruits produced (crop sizes) by trees showed positive density dependence in 2011 and 2012 but not in 2013. Finally, the total number of seeds produced did not show DDD effects, indicating that conflicting demographic processes can disrupt the initial spatial pattern of tree investment in reproduction.

4. Synthesis: Understanding complex spatial effects of density- and distance-dependent (DDD) processes requires dissection of component processes to attain the complete picture since contrasting DDD processes may be hidden behind a single cumulative measure of reproductive success. The combination of novel and classic mark correlation functions used here constitute a powerful spatially-explicit tool that can be broadly applied to unravel conflicting mechanisms of DDD regulating the persistence of sessile organisms at a range of
spatial scales. Our findings help to explain why some authors failed to find expected DDD of PRS and highlight the importance of detailed multi-year field studies on plant reproductive success.

Key words: aggregation, conflicting effects, density dependence, Doñana National Park, mark correlation functions, plant-animal interactions, population regulation, pollination, plant population and community dynamics, small populations
INTRODUCTION

Long-term persistence of natural populations is critical for the maintenance of community structure, ecosystems functioning, and essential ecosystem services. Both plant and animal populations are frequently regulated by density and distance dependence of their demographic parameters that impose bounds on population size (Strong 1986, Hixon et al. 2002). Contrasting density- and distance-dependent processes can operate during the often intricate life cycles of organisms sequentially or even simultaneously (Roughgarden et al. 1988). Diverse proximate mechanisms of density and distance dependence can thus act during different ontogenic stages, spatial scales, and contrasting directions (e.g. positive vs. negative density-dependence; see Courchamp et al. 2008 for a review).

In plant populations, the spatial distribution of individuals determines how a species uses resources, how it is used as a resource, and how it reproduces (Condit et al. 2000, Wiegand et al. 2007, Fedriani et al. 2010). For instance, isolated individuals are less often visited by effective pollinators and thus produce fewer fruits than more aggregated individuals (i.e. positive density-dependence; Nielsen & Ims 2000, Aizen & Vázquez 2006, Fox 2007). Such negative effects of isolation on fruit initiation can be amplified or attenuated by density dependence in other demographic processes acting on subsequent plant life stages (e.g., fruit development). In particular, high conspecific density may either facilitate encounter and exploitation by seed predators and pathogens (Janzen 1970) or promote predator satiation and thus attenuate the per capita chances of predation (Augspurger 1981).

Competition for resources or pollinators can also lower fitness in high-density neighborhoods (Harper 1977, Spigler & Chang 2008, Gunton & Kunin 2009). Recent investigations in self-incompatible species have shown that negative density- and/or distance-dependence is most
likely when mating tends to occur among highly related neighbors, which results in low
good quality of available pollen and lessened plant reproductive success (hereafter, PRS) (Ishihama
et al. 2006, Aizen & Harder 2007). Whether plant aggregation has an overall net positive or
negative effect on individual fitness or whether partial effects cancel each other is an
important question necessary to fully understand how plant populations are regulated. The
answer requires the identification of potentially conflicting density- and distance-dependent
(hereafter, DDD) effects that act upon different life stages, and may also act over different
spatial scales within a population. Like other ecological and evolutionary processes (Levin
1992, Peterson et al. 1998, Thompson 2013), density and distance dependence of PRS vary
with spatial scale and the distance between neighboring conspecifics. Surprisingly, however,
only a few studies have investigated such processes in a spatially-explicit way across spatial scales
and on multiple components of plant reproduction (Knight et al. 2005, Gunton & Kunin 2009,
Gómez et al. 2009). Spatially-explicit approaches are needed to reveal the complexity of
density and distance dependent effects that operate simultaneously over multiple spatial scales
and reproductive success components.

The analysis of marked spatial point patterns (Illian et al. 2008, Wiegand & Moloney 2014)
constitutes a powerful, albeit rarely used approach in ecology that allows identification of
distance- and density-dependence of PRS over a range of spatial scales. This technique
involves statistical analysis of mapped point patterns (e.g., reproductive plants) and additional
proprieties of the plants, called "marks" that represent, for example, PRS components (e.g.,
fruit initiation and development successes, number of seeds per fruit, etc). Analysis of such
quantitatively marked patterns allows detection of correlations in PRS across a range of
spatial scales (e.g., Illian et al. 2008, Getzin et al. 2011, Raventós et al. 2011, Wiegand et al.
2013, Fedriani & Wiegand 2014). This allows us to find out if, for example, whether
pollination success is larger for trees that have more neighbors compared to trees with fewer
neighbors. For detecting such density-dependent effects we specifically tailored a function that directly measures the correlation between plant density and PRS. This new “density correlation function” $D_{mk}(r)$ estimates the correlation coefficient between the reproductive success of all trees and the density of neighbors located within distance $r$ of a specific tree.

In this study, we investigate the occurrence of possibly conflicting DDD processes in different components of pollination and fruiting success of the self-incompatible Iberian pear *Pyrus bourgaeana* in a Mediterranean area of southern Spain. Our data comprise three consecutive reproductive seasons. The low-density *P. bourgaeana* population studied here experiences severe seed dispersal limitation that leads to small-scale aggregated establishment beneath reproductive trees and eventually to adult tree clustering (Fedriani et al. 2010). These tree clusters are readily located and exploited by diverse pollinators (Żywiec et al. 2012). Therefore, we hypothesized that aggregated trees would experience higher pollination success (i.e. fruit initiation) than isolated trees which are more difficult to locate by pollinators. Also, because most recruitment occurs near parent trees and high-density neighborhoods tend to be composed by highly related individuals (Rodríguez 2009), we predicted more fruit abortion and lower fruit development (i.e. fruits that complete development) for trees with close neighbors than for isolated trees (Herlihy & Eckert 2004, Aizen & Harder 2007). More specifically, we seek to answer the following three questions: 1) Do measures of relative reproductive success (percentage of flowers that set and develop fruit) show DDD effects and, if so, at what spatial scale? 2) What is the net effect of density and distance among neighbors on absolute measures of reproductive success of *P. bourgaeana* (i.e., crop size, total number of seeds per tree)? and 3) Are the DDD effects of different reproductive components consistent in sign and spatial scale, and among reproductive seasons? To answer these questions, and to progress our ability to unravel density from distance dependent mechanisms acting on PRS, we use standard mark correlation statistics in combination with a new density
correlation function that quantifies the correlation between a mark of the plants (e.g. a measurement of PRS) and the number of conspecific neighbors within a given distance $r$.

MATERIALS AND METHODS

Study system

*Pyrus bourgaeana* (Rosaceae) is a small tree distributed across the southern Iberian Peninsula and northern Morocco (Aldasoro et al. 1996). Our focal population is located in Doñana National Park (510 km$^2$; 37°89′N, 6°26′W), on the west bank of the Guadalquivir River estuary in southwestern Spain. The climate is Mediterranean sub-humid, characterized by dry, hot summers (June-September) and mild, wet winters (November-February). In the Doñana area, this tree population occurs at low densities (< 1 ind/ha) across the landscape but tree local distribution varies from highly isolated to strongly aggregated (6-8 reproductive individuals within ~25 m; Fedriani et al. 2010). The establishment and persistence of such tree clusters arises from dispersal limitation and spatial contagion of dispersed seeds (Fedriani & Wiegand 2014). These processes create considerable relatedness among individuals within clusters (Rodriguez 2011). The fieldwork (see below) was undertaken in an irregular quadrilateral plot of 49-ha in which the locations of all *P. bourgaeana* reproductive trees were known.

*Pyrus bourgaeana* is a self-incompatible species that flowers during February-March and is pollinated by a diverse insect assemblage (Żywiec et al. 2012). Flowers are hermaphrodite, showing radial symmetry and usually five oval petals. Fruit crop size strongly varies among individuals and seasons, though each individual usually produces 100-500 fruits that ripen during fall. Mature fruits are globose pomes, comprising 1-5 viable seeds. Pre-dispersal seed predation by invertebrates is generally low (<5%; Fedriani & Delibes 2009). However,
during the long period of fruit ripening (March-September) a considerable fraction of the
initial fruit set fails before completing development (Authors unpublished data).

Fruit set, crop size, and total seed number

Since P. bourgaeana trees produce many inflorescences and reproductive success often varies
within trees (Herrera 2009), among each pollination season (i.e. 2011, 2012, and 2013) and
for each reproductive individual, we monitored several inflorescences to ensure that we attain
a good representation of its reproductive success. Specifically, we selected 2-6 branches of
each flowering P. bourgaeana tree (n = 71, 67, and 67, respectively) within the study plot
(most of the 73 trees flowered during all three seasons). On each branch, we tagged from 1-4
inflorescences, recorded the number of flowers per inflorescence, and monitored them once
per month until complete fruit fall (i.e. early December). During 2011, our monitoring started
in early May (i.e. after anthesis) and thus we marked 142 infrutescences with 440 recently set
fruits. Thus, for this season, we estimated fruit development (i.e. number of fruits that
completed their development until early September / number of set fruits). During 2012 and
2013 monitoring started early in March before anthesis; we monitored the fate of 5012 and
5048 flower buds placed in 529 and 531 inflorescences, respectively. For both seasons, we
estimated both fruit initiation (i.e. number of flowers that set fruit / initial number of flowers)
as well as fruit development (as defined above). Overall fruit set (fully developed fruits per
flower) for each of these seasons was then estimated as the product of fruit initiation and fruit
development successes. Though overall fruit set best describes female performance of
individual trees, distinguishing between fruit initiation and development at a range of spatial
scales can help to identify density dependent mechanisms acting on individual fitness
components.
During the three seasons, just before the initiation of fruit fall in early September, we estimated the crop sizes of all reproductive trees within the study plot by counting their fruits with the aid of binoculars, when needed. In *P. bourgaeana*, fruits are arranged in discrete clusters which facilitated estimation of reliable crop size by visually counting fruits from the ground (e.g., Janzen et al. 2008). Finally, during 2011, we estimated the total number of seeds produced by each focal tree by quantifying average seed number per fruit (three fruits per tree) and multiplying it by the corresponding crop size (e.g. Spligler & Chang 2008).

**Mark-correlation functions**

To detect DDD effects on different components of *P. bourgaeana* PRS, we used techniques of marked point pattern analysis (Illian et al. 2008; Wiegand & Moloney 2014). One data set comprises for each reproductive *P. bourgaeana* tree *i* the coordinates *x*, and given measure *m*, of reproductive success that can be in our case percentage of flowers that set and develop fruit, crop size, or the number of seeds per tree. This data structure is a univariate quantitatively marked point pattern (Illian et al. 2008, Wiegand & Moloney 2014) where the coordinates *x* represent the univariate point pattern and the measure of reproductive success *m* represents a quantitative mark.

Mark correlation functions are based on all (ordered) pairs of trees which have inter-point distances within the small interval (*r - h, r + h*). The parameter *h* is called bandwidth and must be wide enough to yield a sufficient number of pairs in each distance class *r* but small enough to reveal relevant biological detail (Illian et al. 2008). The basic idea of mark correlation functions is then to estimate the mean value *c*(r) of a test function *t*(*m*, *m*) of the two marks *m*, and *m*, taken over all (ordered) pairs *i-j* of trees which have inter-point distances of *r ± h*. This procedure is then repeated for a range of distances *r* taken at distance steps *Δr* to obtain a non-normalized mark correlation function *c*(r) (Illian et al. 2008). To obtain the final mark...
correlation function, the $c_t(r)$ is normalized with the expectation $c_t$ of the test function taken over all pairs of trees, irrespective on their spatial separation:

$$k_t(r) = c_t(r)/c_t. \quad (1)$$

Many different test functions $t(m_i, m_j)$ are possible (Illian et al., 2008). We use here the r-mark correlation function $k_m(r)$ which is based on the test function

$$t(m_i, m_j) = m_i. \quad (2)$$

An estimator of the corresponding non-normalized mark correlation function is given by

$$\hat{c}_j(r) = \hat{\mu}(r) = \frac{\sum_{i=1}^{n} \sum_{j=1,i\neq j}^{n} m_i \times k_h(\|x_i - x_j\| - r)}{\sum_{i=1}^{n} \sum_{j=1,i\neq j}^{n} k(\|x_i - x_j\| - r)} \quad (3)$$

where the “box kernel” function $k_h(d)$ yields a value of $1/2h$ if the two trees with coordinates $x_i$ and $x_j$ have inter-point distances of $r \pm h$ and zero otherwise (Illian et al. 2008; Wiegand & Moloney 2014). Thus, the denominator of equation (3) yields the number of ordered pairs of trees i and j which are distances of $r \pm h$ apart and therefore, equation 3 yields the mean value of the mark $m_i$ of the first tree i of these pairs. The normalization constant $c_t$ of the r-mark correlation function which is taken over all pairs of points, regardless of their spatial separation, is obtained by replacing in equation 3 $k_h(d)$ by $1/2h$:
\[ \hat{c}_j = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} m_i / 2h}{\sum_{i=1}^{n} \sum_{j=1}^{n} (1/2h)} = \frac{1}{n} \sum_{i} m_i = \mu \quad (4) \]

and yields \( \mu \), the mean value of \( m_i \) taken over all trees \( i \) (Illian et al. 2008).

Thus, \( k_m(r) > 1 \) indicates that the PRS of trees that have neighbors at distance \( r \) is on average larger than the average PRS, indicating a positive effect of nearby \( P. bourgaeana \) trees on PRS. Conversely, \( k_m(r) < 1 \) indicates that the PRS component of trees that have neighbors at distance \( r \) is smaller than the average PRS, indicating a negative effect of nearby trees on PRS (for further methodological details see Illian et al. 2008 and Wiegand & Moloney 2014).

We also used a mark correlation function that characterizes the spatial covariance in PRS of two \( P. bourgaeana \) trees separated by distance \( r \). The appropriate test function for this purpose was proposed by Schlather et al. (2004):

\[ t(r, m_i, m_j) = [m_i - \mu(r)][(m_j - \mu(r)] \quad (5) \]

which results in a Moran’s \( I \) like summary statistic \( I_{mm}(r) \), a spatial variant of the classical Pearson correlation coefficient (Shimatani 2002). \( I_{mm}(r) \) is normalized by the mark variance \( \sigma^2 \). Thus, the \( I_{mm}(r) \) is the straight forward Pearson correlation coefficient between the two variables \( m_i \) and \( m_j \) defined by the ordered \( i-j \) pairs of trees separated by distance \( r \pm h \). Note that a test function that adjusts for the mean \( \mu(r) \) that considers only pairs of trees separated at a given distance \( r \), not the population mean \( \mu \), is required to yield a summary statistic with the interpretation of a correlation coefficient (Schlather et al. 2004).
Finally, we developed a new function that directly relates the PRS component of a P. bourgaeana tree to the density of its conspecific neighbors. This “density correlation function” $C_{m,K}(r)$ estimates the classical Pearson correlation coefficient between the reproductive success $m_i$ of a tree and the number of neighbors within distance $r [=\lambda K_i(r)]$. Thus, the density correlation function is based on the following test function:

$$t(r, m_i, K_i) = [m_i - \mu][\lambda K_i(r) - \lambda K(r)],$$

where $m_i$ is the PRS of the focal tree $i$, $\mu$ is the mean PRS of the population, $\lambda$ the overall density of trees in the study area, $\lambda K_i(r)$ the number of neighbors around the focal tree $i$ within distance $r$, and $\lambda K(r)$ the mean number of neighbors within distance $r$ for all trees. Because correlation functions are invariant under multiplication with a constant it does not matter if we use absolute number of neighbors (e.g., $\lambda K(r)$) or density of neighbors ($\lambda K(r)/\pi r^2$).

The $K_i(r)$ is basically a “local” $K$-function and the average over all $K_i(r)$’s yields the well known $K$-function $K(r)$. Estimation of $K_i(r)$ requires an edge correction factor $w_i$ if the neighborhood around tree $i$ is not fully within the observation window, which yields $\pi r^2/A_i$ where $A_i$ is the area of the disk around point $i$ within the observation window. The density correlation function $C_{m,K}(r)$ is normalized by the product of the standard deviations $\sigma_m \sigma_K$ of the marks $m_i$ and the individual $K$-functions $K_i(r)$, respectively. “C” stands for correlation, “$m$” for the first mark $m_i$, and “$K$” for the second mark $K_i(r)$. In Appendix S1 we use artificial data sets to test the ability of the density correlation function to reveal different known DDD effects (see Fig. S1-S2).

To test whether the data show spatial correlations in the marks, we contrast the three mark correlation functions of the data to that of repeated simulations of a null model without spatial
correlation in the marks. This null model was implemented by randomly shuffling the marks over all *P. bourgaeana* trees (Illian et al. 2008; Wiegand et al. 2013). We conducted 199 randomizations to estimate simulation envelopes for our summary statistics \( k_m (r) \), \( I_{mm}(r) \), and \( C_{m,K}(r) \), being the 5th lowest and highest values of the summary statistics among the 199 simulations of the null model (e.g., Wiegand et al. 2013, Fedriani & Wiegand 2014). We observe a departure from the null model at particular distances \( r \) if the summary statistic of the observed data is outside the simulation envelopes.

To test the overall fit of the random marking null model over a range of distances we used the “Diggle-Cressie-Loosmore-Ford” Goodness-of-Fit (GoF) (Baddeley et al. 2014) for the entire distance interval up to 50m. The GoF test returns a \( P \)-value that indicates significant departures of the observed mark correlation function from the random marking null model over the distance interval of interest. If a significant departure occurs, we can inspect the plot of the mark correlation function together with the simulation envelopes to identify the specific distances \( r \) where departures occurred.

We estimated the two mark correlation functions and the pair correlation function every five metres up to distances of 50m, where the distance bins centered on \( r = 2.5m, 7.5m, 12.5m, \ldots \) included all pairs of trees within distance \((r - h, r + h)\), being \( h = 2.5m \) the bandwidth. The distance bins for the cumulative density correlation function yield \( r = 5m, 10m, 15m, \ldots \) All point pattern analyses were conducted with the software *Programita* (Wiegand & Moloney 2014) available at http://programita.org/

**RESULTS**

*Aggregation of P. bourgaeana trees*
We first estimated the degree of clustering of our focal tree population. The non-cumulative neighborhood density varied with distance from focal trees. Within 5-10 m away from a focal tree, it was on average 29 times higher than expected by a completely random pattern, at 15-20 m it was still 12 times higher, but beyond 40 m it approximated the overall density \( \lambda \) (see Appendix S1). As expected, the cumulative relative neighborhood density \( K(r)/(\pi r^2) \) declined somewhat slower and approximated the overall density \( \lambda \) at radius \( r \) of about 100 m (Fig. S3).

Thus, we expect the strongest density-dependent effects to occur within 20-30 m which should slowly fade away after 40-50 m (see Fedriani et al. 2010 for a more details on \( P. \) bourgaeana clustering).

**Fruit initiation**

Fruit initiation during 2012 and 2013 was low (12% and 8.5% on average) and highly variable (range 0-40%) among \( P. \) bourgaeana trees during both years (Table 1). As expected, the r-mark-correlation function for the percentage of flowers that initially set fruits during 2012 was significantly higher for individuals with conspecific neighbors within 35 m (Fig 1A; \( P = 0.01 \)). For example, a tree that had other trees at distance 25 m set about 1.4 times more fruit than the mean of all trees in the study area, and such differences declined quickly for above 35 m (Fig. 1A). Schlather’s \( I_{mm} \) correlation function showed that fruit initiation of trees separated by less than 20 m were positively correlated (Fig 1B; \( P = 0.015 \)). The density correlation function \( C_{m,K} \) indicated a highly significant and positive density dependence at tree neighborhoods > 5 m (\( P < 0.01 \); Fig. 1C) with a peak at 30m \( [C_{m,K}(r) = 0.5] \). The 2013 data exhibited very similar patterning, i.e. positive DDD of fruit initiation that was somewhat stronger than that of the previous season (Fig. 1D-F).
Fruit development

Fruit development rate from the total number of flowers that had set fruits (i.e. 440, 579, and 541 in 2011, 2012, and 2013, respectively) was over 3-fold higher than fruit initiation rate, but also very variable among reproductive trees during the three seasons (Table 1). As predicted, and in contrast with the observed trend for fruit initiation, fruit development showed negative density dependence during two of the three years. During 2011, fruit development was significantly lower for individuals with neighbors within 50 m (Fig 2A; P < 0.01). For example, the mean r-mark-correlation value for fruit development of trees with neighbors at 30 m was only about 0.54 times that of the population mean fruit development (Fig. 2A). The spatial correlation in fruit development of two trees r apart was higher than expected for individuals with conspecifics up to 50 m away (Fig 2B; P < 0.01). This was caused by the consistently lower fruit development of nearby trees as shown by the r-mark correlation function. As a consequence we observed strong negative density dependence with maximal correlations between 40 and 80 m (P < 0.01) where trees within clusters showed lower than expected fruit development (Fig. 2C).

During 2012 the trend in fruit development was the same, being lower for *P. bourgaeana* individuals with close conspecific neighbors, though the observed values were not significant and fall within the simulation envelops (Fig 2D-F). During 2013, the r-mark correlation function indicated that fruit development was again significantly lower for individuals with conspecific neighbors up to scale of 40 m (P = 0.04; Fig. 2G). Also, fruit development exhibited a significant negative density dependence at neighborhoods with radius larger than 10m (P = 0.015; Fig. 2I).

Overall fruit set
Overall fruit set of *P. bourgaeana* trees during 2012 and 2013 (0.054±0.006 and 0.028±0.007 fully developed fruits per flower, respectively) was generally low and variable among reproductive trees (Table 1). Interestingly, all three summary statistics during both seasons were always non-significant and fall within the simulated envelops (always, *P* > 0.250; Fig. 3A-C [2012], Fig. 3 D-F [2013]). This result indicates lack of distance and density dependence of overall fruit set as well as lack of spatial correlation among tree in overall fruit set, thus the positive density dependence of fruit initiation was just neutralized by the negative density dependence of fruit development.

**Crop size and total seed number per tree**

Fruit crop size varied extensively among *P. bourgaeana* trees during the three seasons, ranging over three orders of magnitude (Table 1). On average, trees produced twice as many fruits during 2011 as during 2013 (Table 1). Crop sizes exhibited similar spatial patterning during 2011 and 2012, but differed in 2013 (Fig. 4) when fruit crops were smallest. During 2011 and 2012 individuals with nearby conspecific neighbors produced more fruits than average (i.e., up to 30-40 m; Fig. 4A [2011], Fig. 4D [2012]), with differences being significant in 2011 (*P* = 0.015). During 2011, fruit production was subject to significant positive density dependence at scales greater than 10m (*P* < 0.01; Fig. 4C). During 2012, the pattern of density dependence was very similar to that in 2011, but somewhat weaker (*P* = 0.015; Fig. 4F) and restricted to scales from 20-60 m. In contrast, during 2013, all three summary statistics indicated no spatial structures in crop sizes at any smaller spatial scale (*P* > 0.44; Fig. 4G-I).

During 2011, the estimated total number of seeds per reproductive *P. bourgaeana* tree varied widely (i.e. up to two orders of magnitude; Table 1). However, all three summary statistics indicated a general lack of DDD effects on total number of seeds (*P* > 0.145; Fig. S4A-C).
These results indicate that the strong effect of conspecific neighbors at small scales for crop size (Fig. 4) did not influence the number of seeds per fruit. This is probably caused by trees with larger crops that produce fruits with fewer seeds, though such a trend was only marginally significant ($r_s = -0.207, P = 0.096$).

**DISCUSSION**

We used detailed data from three consecutive seasons to examine DDD effects on sequential PRS components over a range of spatial scales. Application of the framework of marked point pattern analysis allowed us to answer our three guiding questions. First, fruit initiation, fruit development and crop size showed DDD effects over spatial scales that correspond to that of the highest neighborhood density of *P. bourgaeana* trees (some 30-40m, somewhat smaller than the diameter of the typical cluster which yields 44m; Fedriani et al. 2010) as indicated by the maximal correlation between reproductive success and neighborhood density. Second, we found the surprising result that the net effect of DDD on the overall fruit set of *P. bourgaeana* trees just canceled because DDD effects on its two components (i.e., fruit initiation by fruit development) acted on opposite directions and with similar strengths. Finally, though the DDD effects of different reproductive components of *P. bourgaeana* were conflicting in sign (fruit initiation and crop size showed positive DDD effects whereas fruit development showed negative DDD effects), they showed similar critical scales imprinted by the strong clustering of the trees. However, DDD effects did not appear every year.

**Conflicting DDD processes**

Density and distance dependence in demographic parameters may be critical for the regulation of many plant populations and thus for ecosystem functioning (Courchamp et al. 2008 review). Few studies, however, have considered DDD effects on several fitness components simultaneously over a range of spatial scales which, as we illustrate, is needed
given that both sort of effects can emerge at contrasting plant life stages and spatial scales (Aizen & Harder 2007). Our results provided clear evidence that different DDD processes affect different aspects of fruit production (Aizen & Vazquez 2007, Gómez et al. 2009, Jones & Comita 2010). First, as expected, *P. bourgaeana* individuals with nearby neighbors (up to 40 m) initiated more fruits during both seasons studied, which is consistent with results from other temperate and tropical species, including some hermaphrodite species (e.g., Kunin 1993, Aizen 1997; but see Spigler & Chang 2008). Such findings are generally attributed to the attraction of pollinators to larger floral displays, and increased pollen transfer per visit at high conspecific densities (Ishihama et al. 2006, Aizen & Harder 2007).

However, fruit development showed negative density and distance dependence during two out of the three monitored seasons (Fig. 2). One hypothesis to explain this result is that most mating occurred between highly related *P. bourgaeana* individuals (e.g., Ishihama et al. 2006, Jones & Comita 2008). In entomophilous species pollen deposition from genetically related donor conspecifics can strongly contribute to qualitative pollen limitation given that insect pollen typically disperse pollen locally (Harder & Barrett 1996, Aizen & Harder 2007). Though the initiation of fruit set can occur despite low-quality pollen, such developing fruits are likely to abort because embryos homozygous for deleterious alleles tend to die during development (Herlihy & Eckert 2004, Aizen & Harder 2007). Several lines of evidence support this hypothesis in our system. Genetic analyses indicates strong relatedness among individuals within clusters (Rodríguez 2009), which is consistent with limited seed dispersal and spatial contagion of dispersed *P. bourgaeana* seeds (Fedriani et al. 2010, Fedriani & Wiegand 2014). Such local genetic structure also occurs in other tree species experiencing limited seed dispersal (Dutech et al. 2002, Ng et al. 2004, Voigt et al. 2009). Moreover, thorough hand-pollination experiments revealed that *P. bourgaeana* flowers supplemented with pollen from individuals away from the focal cluster produced more fruits than...
individuals supplemented with pollen from neighboring conspecifics (Fedriani et al. 2012).

Comparing levels of inbreeding in fully developed versus aborted seeds is a pending relevant task (Jones & Comita 2008)

Further influential factors

Other factors, such as availability of soil resources, intraspecific competition, and tree size could potentially affect fruit initiation and development, and hence overall fruit production (Pacala & Silander 1985, Obeso 2002, García-Camacho et al. 2009). However, we did not find any effect of tree size (i.e. dbh and crown area) on fruit production and the locally dominant sandy substrate appears homogeneous across our study area (Fedriani & Delibes 2009). Furthermore, by sampling all reproductive individuals within our study plot and performing detailed analyses at a range of spatial scales, we have minimized any potential effect of spatial heterogeneity, thus providing robust results. On the other hand, though it cannot be ruled out that high competition for resources and pollinators in high density neighborhoods lessened tree reproductive success, high pollinator visitation and relatively high initial fruit sets and crop sizes are often observed in these tree clusters. Nonetheless, at least during 2011, although individuals in large clusters produced more fruit (Fig. 4) their seed production (a more pertinent aspect of female reproductive success) did not show such marked positive DDD pattern. This result indicates that although fruit production may depend on flower production, subsequent conflicting DDD processes (including intraspecific competition) can modify the spatial pattern of tree reproductive investment.

Conclusions

Our study revealed that complex scale-dependent processes operate at different components of pollination and fruiting success of the self-incompatible Iberian pear Pyrus bourgaeana in a Mediterranean area of southern Spain. Interestingly, DDD processes for sequential tree
reproductive success components operated in opposition and neutralized each other. This result may help to explain why some authors failed to find expected DDD of plant reproductive performance (see Ghazoul 2005 review). Understanding of the complex spatial effects of DDD processes requires thus dissection of component processes to obtain the complete picture since contrasting DDD processes may be hidden behind a single cumulative measure of reproductive success.

Our approach can be widely applied to mapped plant populations for which detailed data on reproductive success are available or attainable. Furthermore, point pattern analyses similar to those presented here could be conducted using reproductive success components concerning subsequent plant stages (e.g. dispersed seeds, seedlings, saplings; Fedriani & Wiegand 2014, Jacquemyn et al. 2010, Wiegand & Moloney 2014) and thus foster understanding of population regulation. Because DDD effects concern many active areas of ecological and evolutionary research (e.g., humanization, invasive species, density-dependent selection; Courchamp et al. 2008, Thompson 2013), the combination of novel and classic mark correlation functions presented here could be broadly applied to unravel conflicting mechanisms regulating the persistence of sessile organisms at a range of spatial scales.

ACKNOWLEDGEMENTS

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21926/BOS) supported this study. JMF was funded by a Marie Curie Intra-European fellowship (FP7-PEOPLE-2011-IEF-298137) and TW by the ERC advanced grant 233066. We do not perceive any potential source of conflict of interest influencing our objectivity in the current investigation.

REFERENCES


Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Test of the density correlation function with artificial data

Figure S1. Analysis of the spatial pattern of adult *P. bourgaeana* trees.

Figure S2. The mark correlation functions for total seed number produced by target *Pyrus bourgaeana* trees during 2011

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Table 1: Sample sizes, mean, standard error, minimum, and maximum values of different *Pyrus bourgaeana* reproductive success components used in our marked point pattern spatial analyses for each of the three years (2011, 2012, and 2013) encompassed during the study. $N_1$, summation of flower buds, seeds, or fruits monitored to estimate fruit initiation and fruit development, as well as total seed numbers. Note that fruit set is given as a proportion. $N_2$, number of reproductive trees considered for the estimation of each reproductive success component.

<table>
<thead>
<tr>
<th>Year</th>
<th>Component</th>
<th>$N_1$</th>
<th>$N_2$</th>
<th>Mean</th>
<th>SE</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>Fruit initiation</td>
<td>-</td>
<td>440</td>
<td>71</td>
<td>0.595</td>
<td>0.047</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>Fruit development</td>
<td>579</td>
<td>61</td>
<td>0.455</td>
<td>0.046</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2011</td>
<td>Overall fruit set</td>
<td>-</td>
<td>5012</td>
<td>67</td>
<td>0.054</td>
<td>0.006</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>Crop size</td>
<td>36215</td>
<td>71</td>
<td>510.07</td>
<td>57.69</td>
<td>16</td>
<td>1858</td>
</tr>
<tr>
<td>2011</td>
<td>Total seed number†</td>
<td>78064</td>
<td>63</td>
<td>1239.1</td>
<td>188.2</td>
<td>59</td>
<td>7263</td>
</tr>
<tr>
<td>2012</td>
<td>Fruit initiation</td>
<td>5012</td>
<td>67</td>
<td>0.120</td>
<td>0.011</td>
<td>0</td>
<td>0.345</td>
</tr>
<tr>
<td>2012</td>
<td>Fruit development</td>
<td>579</td>
<td>61</td>
<td>0.455</td>
<td>0.046</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2012</td>
<td>Overall fruit set</td>
<td>5012</td>
<td>67</td>
<td>0.054</td>
<td>0.006</td>
<td>0</td>
<td>0.190</td>
</tr>
<tr>
<td>2012</td>
<td>Crop size</td>
<td>17434</td>
<td>67</td>
<td>257.75</td>
<td>37.76</td>
<td>0</td>
<td>1448</td>
</tr>
<tr>
<td>2013</td>
<td>Fruit initiation</td>
<td>5048</td>
<td>67</td>
<td>0.085</td>
<td>0.011</td>
<td>0</td>
<td>0.399</td>
</tr>
<tr>
<td>2013</td>
<td>Fruit development</td>
<td>541</td>
<td>50</td>
<td>0.276</td>
<td>0.044</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2013</td>
<td>Overall fruit set</td>
<td>5048</td>
<td>67</td>
<td>0.028</td>
<td>0.007</td>
<td>0</td>
<td>0.351</td>
</tr>
<tr>
<td>2013</td>
<td>Crop size</td>
<td>15725</td>
<td>72</td>
<td>218.40</td>
<td>30.58</td>
<td>0</td>
<td>1249</td>
</tr>
</tbody>
</table>

†Estimated only during the 2011 fruiting season.
FIGURE LEGENDS

Figure 1: Fruit initiation. The three mark correlation functions (r-mark correlation function, Schlather’s $I$ function, and density correlation function) to detect a potential spatial structure in *P. bourgaeana* fruit initiation during 2012 (A-C) and 2013 (D-F). The r-mark correlation function is the mean reproductive success component (i.e., fruit initiation) $m_i$ of a tree at distance $r$ of another tree, Schlather’s $I_{mn}$ correlation function is the correlation between the fruit initiation $m_i$ and $m_j$ of two trees $i$ and $j$ separated by distance $r$, respectively, and the neighborhood correlation function $C_{mk}(r)$ is the correlation between the mark $m_j$ and the number of neighbors within distance $r$. The observed mark correlation functions (closed disks), the expected function under the null model of random mark $i$ (grey solid line), and the corresponding simulation envelopes (solid lines), being the 5th lowest and highest values of the functions created by 199 simulations under the null model, are shown. To test the overall fit of the random marking null model over a range of distances we used Goodness-of-Fit (Baddeley et al. 2014) for the entire distance interval up to 50m. The GoF test returns a $P$-value (shown in each panel) that indicates significant departures of the observed mark correlation function from the random marking null model over the distance interval of interest.

Figure 2: The mark correlation functions for fruit development in *Pyrus bourgaeana* during 2011 (A-C), 2012 (D-F), and 2013 (G-I). Other conventions are as described in Figure 1.

Figure 3: The mark correlation functions for the overall fruit set (i.e. the product of fruit initiation by fruit development) in *Pyrus bourgaeana* during 2012 (A-C) and 2012 (D-F). Other conventions are as described in Figure 1.
Figure 4: The mark correlation functions for crop size in *Pyrus bourgaean* during 2011 (A-C), 2012 (D-F), and 2013 (G-I). Other conventions are as described in Figure 1.
Figure 1.
Figure 2.
2012

A. \( P = 0.66 \)

B. \( P = 0.25 \)

C. \( P = 0.32 \)

2013

D. \( P = 0.85 \)

E. \( P = 0.85 \)

F. \( P = 0.74 \)

Figure 3
Figure 4