David Draper & José María Iriondo

Spatial patterns of vegetative and sexually reproducing individuals of *Marsilea strigosa* Willd.

Abstract

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We analyzed the spatial distribution of *Marsilea strigosa*, an aquatic fern, considering two life stages (vegetative and sexual) and interpreted results taking into account possible facilitation or competition effects among individuals as well as existing colonization strategies. Marsilea strigosa is distributed from Spain to the Black Sea. It is considered a rare species due its high habitat specificity and small fragmented populations. We surveyed 95% of the Balearic population of *M. strigosa*, covering a surface of approximately 1000 m2. Single life stage and bivariate distribution patterns were analyzed using second-order spatial analysis based on Ripley's K-function. The relationship between vegetative and sexual node types is discussed. Aggregation of vegetative and sexual nodes was the only spatial point pattern found. The same results were obtained when both stages were analyzed together with bivariate function. We discuss the effect of life stage on the balance between competition and facilitation in dynamic environments. The importance of these two processes in colonization sites can best be understood by comparing them between different life stages.

Introduction

Marsilea strigosa is an amphibious rhizomatous geophyte (Boudrie 2004). It can produce very slender runners (2-25 cm long) that bear leaves and roots at each node. The nodes have a high capacity of vegetative reproduction, while some also produce macroscopic reproductive structures (3-5 mm) which can be easily identified during the dry season. The roots need at least a thin layer of clay.

This aquatic fern species is scattered from Spain to Crimea. Although it is widely distributed, it presents high habitat specificity, and the population sizes are frequently low. As a result, *M. strigosa* is considered a rare species belonging to Category V of the Rabinowitz (1981) classification. *Marsilea strigosa* is a hydrophyte that requires a combination of geophysical and weather conditions: small depressions in sites where a watershed appears during the rainy season followed by a dry season in which the pond dries up. This cyclic regime is probably one of the most characteristic features of the Mediterranean climate. *Marsilea strigosa* has no soil type restrictions and tolerates dramatic water level changes. The pond depth may however be a limitation. *Marsilea strigosa* can colonize pond sites up to a depth of 0.5 m. Biogeographical history is another factor inherent to this species' present distribution.

The cyclic regime of temporary ponds excludes the occurrence of plants that cannot tolerate the dry period as well as typical grassland species that cannot tolerate the flooding period. *Marsilea strigosa* shares this habitat with a few small annual or some perennial species such as *Mentha cervina* and *Isoetes setacea* (Grillas & al. 2004).

The plant spatial patterns have long been a major issue in ecology (Ashby 1935; Dale 1999; Greig-Smith 1983). The complex combinations of negative and positive interactions between plant species appear to be common in nature, and are not restricted to particular communities or biomes (Walker & Chapin 1987; Chapin & al. 1994; Callaway 1995).

The co-occurring facilitative and competitive effects in the same species occur in other ecosystems and often vary in time or space (Hay 1986; Eldridge & al. 1991; Aguiar & Sala 1994; Belsky 1994; Callaway 1994; Callaway & King 1996; Nicotra 1998), but the factors that determine the positive or negative balance between them are poorly understood. Factors that have been considered include life stage, plant density, specific physiology of the species, indirect interactions, and abiotic stress. The aim of this work was to study the spatial distribution of *M. strigosa* individuals by considering its vegetative and sexually reproductive life stages and to determine whether the production of sexual structures follows a particular pattern in its populations. The question is, do sexual nodes tend to accumulate in specific areas of the pond? Is there repulsion between sexual and vegetative nodes? Is it possible to infer information on the colonization process of the pond from the spatial distribution patterns of sexual and vegetative nodes?

Material and methods

Field sites and sampling method

The sites selected for this study were the temporary pond system of Marina de Llucmajor, Majorca (UTM 31SDD86) and two isolated ponds in Minorca (UTM 31SFE01, 31TEE93). The climate in this area is humid from autumn to mid-spring with a severe dry period in summer. The mean annual precipitation is 450 mm/yr. and mean annual temperature is 17ºC with a soothing effect from the Mediterranean Sea. The sites are almost flat, with karstic and schist soils (Majorca and Minorca respectively) with a sandy-loam texture. Some ground depressions and vegetation gaps accumulate water, allowing the formation of temporary ponds. The surrounding vegetation is mainly typical of the Mediterranean garrigue. The seasonal change in water availability is the main constringent for plant colonization in these systems, occupied by highly specialized flora composed of Mediterranean therophytic and geophytic species. The main herbivores are rabbits and goats.

Six temporary ponds were surveyed in Majorca and two temporary ponds in Minorca to cover major ecological differences. The shape and size of the sampled plots differed from pond to pond, as pond size, *M. strigosa* total population and *M. strigosa* cover varied between ponds (Tab. 1).

Table 1. Code, location in UTM and spatial features of surveyed ponds and plots.

Pond code	UTM	Island	Area surveyed (m^2)	Pond depth (m)	% of population covered	% of pond covered
B2	31SDD8136	Majorca	41	-0.19585	100	100
B3	31SDD8136	Majorca	52	-0.45307	20	30
B4	31SDD7936	Majorca	57	-0.47505	100	20
B20	31SDD8267	Majorca	20	-0.24321	60	15
B51	31SDD8359	Majorca	42	-0.39954	100	100
B61	31SDD8361	Majorca	19	-0.29735	40	30
BF	31TEE9834	Minorca	49	-0.17345	100	100
BV	31SFE0919	Minorca	803	-0.28375	50	50

The table shows the code names that were assigned to each pond. The aim was to survey the entire area of each pond due to the clonal capacity of *M. strigosa*. When this was not possible, a transect was established crossing the point with the deepest water level. Fieldwork was carried out in early summer 2003 when the ponds were totally dry and the development of reproductive structures was completed. The percent of population surveyed was determined considering the surface of the carpet of *M. strigosa* in each pond.

A grid of 1x1 m squares was established on the population using a total station transit (model TPS407, Leica Geosystems AG, CH) to position each corner of the grid. Each node was then marked with a coloured drawing pin to identify its life stage (vegetative or sexual). All grid squares were photographed and the pictures were orthogonally corrected and joined using Idrisi Kilimajaro software. The resulting composition indicated the position and life stage of each marked node (Fig. 1). A land cover layer was also obtained from the final image allowing us to identify three different categories (i.e. area covered by soil, rock and other vegetation).

Statistical analysis

The spatial distribution patterns were analysed by Ripley's *K*-function (Ripley 1976, 1981; Diggle 1983; Haase 1995; Haase & al. 1996). The edge correction was calculated by the weighted method described by Getis & Franklin (1987) and modified by Haase (1995). This approach identifies the scales above which a non-random distribution occurs, and can be used to develop hypotheses about spatial processes at specific scales with the common null model indicating complete spatial randomness (CSR). The null hypothesis was tested for all obtained points together as well as for vegetative nodes and sexual nodes.

To linearize the plot of $K(t)$ against t and stabilize variances, $L(t)$ statistic was used (Diggle 1983): $L(t) = \sqrt{[K(t)/\pi]} - t$. The expected value of $L(t)$ under a Poisson process is 0. Positive values indicate spatial clustering, whereas negative values indicate spatial segregation. When $L(t)$ and the confidence envelope are plotted on the same axes, patterns of clumping and regularity become apparent. If $L(t)$ exceeds the upper confidence interval for any distance class, the points are relatively closer together than expected, indicating clustering or clumping. If $L(t)$ falls below the lower confidence interval for a distance class,

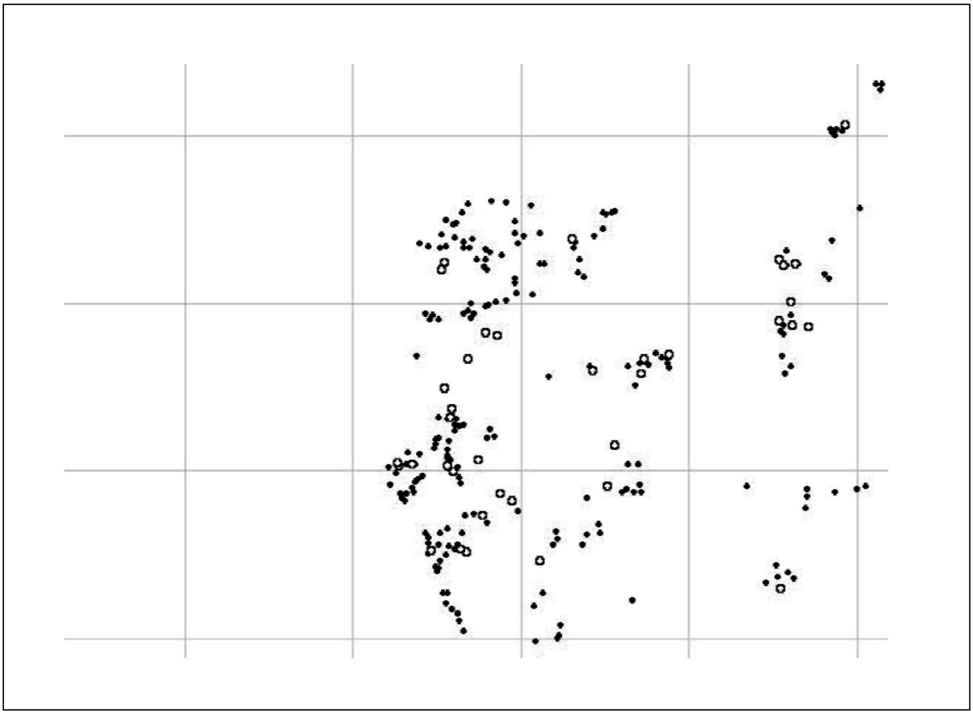


Fig. 1. Example of node distribution corresponding to part of the sample plot of pond BV from Minorca (dots: vegetative nodes; open circles: sexual nodes). Background grid is referred to 1m^2.

the points are relatively farther from one another than expected under a random distribution indicating regularity, repulsion or over-dispersal of points. The values of $L(t)$ within the confidence envelope indicate random distribution of points at those scales (Sharpe 1991). Each study plot was analyzed for distances up to 25% the length of the shortest side.

As Ripley's K, and its derived L statistic are not bound, comparisons of spatial patterns of different species or different study plot sizes should be based on the significant spatial scale. Significance is achieved by using Monte Carlo simulation, which generates n simulations of a Poisson point pattern process that provide a confidence envelope (Parish & al. 1999). This confidence envelope is defined by the extreme maximum and minimum values of the simulation, where 99 simulations correspond to a 0.01 significance level (Ripley 1979). In our study we used a 99% confidence interval.

The bivariate spatial analysis, using the function $L_{12}(t)$ (a transformation of the function $K_{12}(t)$; Ripley 1977), was carried out to test the relationship between the spatial patterns of vegetative and sexual nodes. The combined patterns can suggest spatial attraction, independence, or repulsion (Parish & al. 1999).

The univariate and bivariate analyses were performed using the SPPA (v. 2.0.3. Haase) software package (http://home.t-online.de/home/haasep/sppagree.htm).

Results

The vegetative nodes were generally more common than sexual nodes, accounting for 97% of total nodes in pond BF. The proportion of sexual nodes was only higher in ponds B20 and BV (Tab. 2). The node density seems to be related to lower vegetation cover but does not seem dependent on rock cover. The Minorcan ponds (BF and BV) are characterized by an almost total absence of rocky slopes and these two ponds had the highest percentage of vegetation cover.

When the distribution patterns of vegetative nodes and sexual nodes were analysed separately, $L(t)$ exceeded the upper confidence interval for all distance classes in all ponds indicating a clustering or clumping profile at almost all distances (Fig. 2-9). Three main patterns were observed: a) a steady increase in aggregation with distance (e.g., Fig. 5), b) asymptotic profile (e.g. Fig. 7) and c) presence of a relative maximum (e.g. Fig. 9). Maximum values were obtained at a distance of c. 0.8 m (Fig. 2, 3, 7, 9). In Figure 9 an interval of maximum aggregation can be observed between 1-3 m, followed by a sharp decrease in aggregation which is, nevertheless, still significant.

The bivariate spatial pattern of the two life stages showed a significant trend to aggregation (Fig. 2-9). Lower rates of aggregation were observed in bivariate spatial patterns as compared to univariate spatial patterns (Fig. 2, 3, 5, 8, 9).

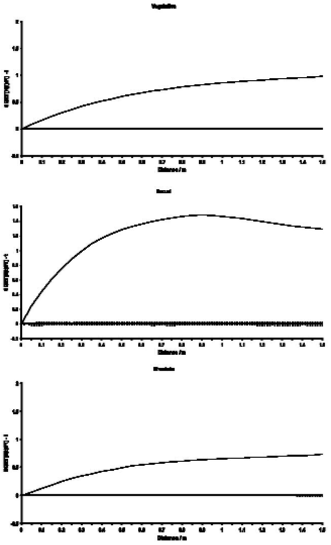
Considering all the sampled patches, exclusive vegetative clumps were common in small patches (<25 nodes), whereas reproductive nodes appeared when node size was ≥ 25.

Discussion

The sexual reproduction is considered to be more “expensive” in terms of resources than vegetative reproduction. Increased vegetative effort is generally associated with stable periods, while a major sexual strategy is considered under non-optimal environmental conditions. This is best represented in pond **BF** where *M. stigosa* covered almost all the flooded area and only 2.31% of the nodes adopted a sexual strategy. On the other hand,

Table 2. Percentage vegetative and sexual nodes, density and percentage rock, vegetation and bare soil cover.

Pond code	% vegetative	% sexual	density nodes/m^2	% rock	% vegetation	% bare soil
B2	88.53	11.47	99.51	47.13	3.84	49.03
B3	79.82	20.18	144.06	41.69	2.26	56.05
B4	59.35	40.65	19.04	26.76	9.33	63.90
B20	40.51	59.49	206.50	17.53	7.19	75.28
B51	72.88	27.12	117.21	97.18	2.75	0.07
B61	60.29	39.71	232.37	17.20	0.00	82.80
BF	97.69	2.31	39.84	1.24	81.12	17.64
BV	38.81	61.19	6.97	0.01	69.42	30.57



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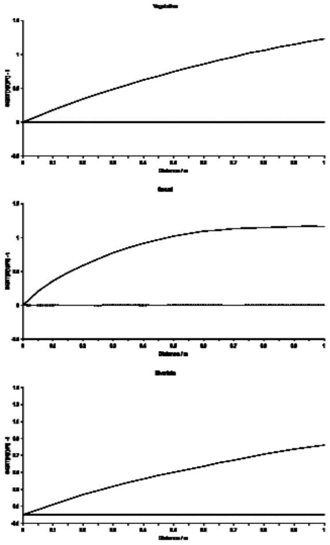

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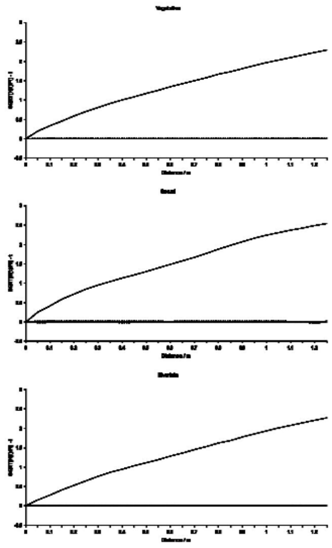

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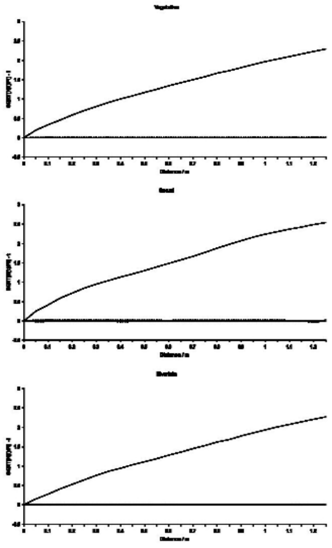

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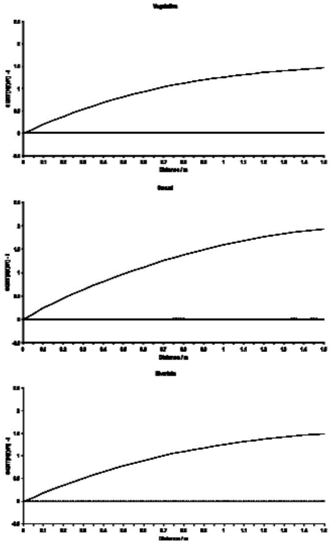
Figs. 2-5. Estimates of second-order neighborhood of ponds B2, B3, B4, B20 respectively. Each pond is analyzed considering a univariate distribution for vegetative and sexual nodes as well the interaction between both types in the bivariated graph. Thick line: observed distribution; Thin lines: 99% confidence envelope (when visible).



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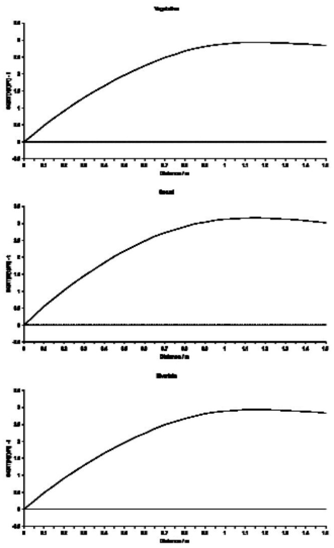

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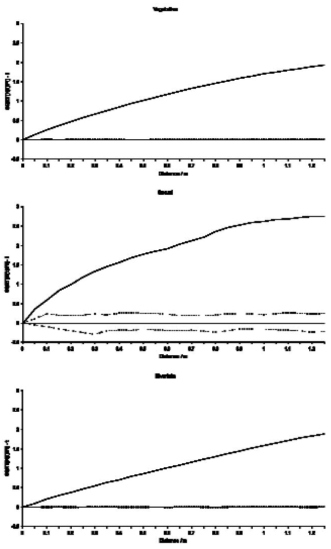

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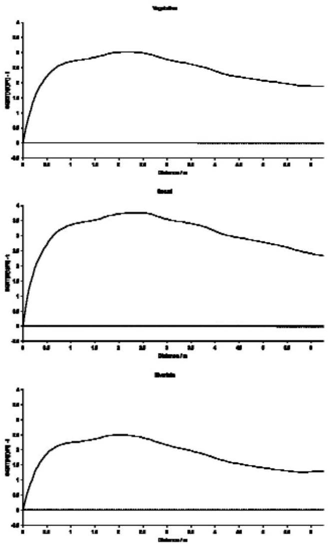

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Figs. 6-9. Estimates of second-order neighborhood of ponds B51, B61, BF, BV respectively. Each pond is analyzed considering a univariate distribution for vegetative and sexual nodes as well the interaction between both types in the bivariated graph. Thick line: observed distribution; Thin lines: 99% confidence envelope (when visible).

pond BV, which had the lowest node density, also had the highest percentage of sexual nodes. Pond BV tended to have a random point distribution pattern due to the gaps between clumps (Fig. 1, 9).

The soil availability and water level seem to be the main limitations in the constantly changing environment of temporary ponds which *M. strigosa* is well adapted to. These important resources may explain the preference for adopting one strategy over the other. Bertness & Callaway (1994) hypothesized that the importance of facilitation in plant communities increases with abiotic stress or consumer pressure, because neighbours buffer one another from extremes in the abiotic environment (e.g., temperature or salinity) and herbivory. Alternatively, they hypothesized that the importance of competition increases when abiotic stress and consumer pressure are relatively low. Although herbivory is not the main focus of this work, the two main predators, rabbits and goats, seem to affect the distribution of *M. strigosa* in quite different ways. Rabbits were observed to produce holes in *Marsilea* carpets, modifying the micro-relief and increasing landscape complexity, while goats mow the aerial parts.

The aggregation pattern observed both in vegetative and sexual nodes results from the clonal growth of *M. strigosa* through the production of runners. Considering each node individually, the vascular connection between nodes can be interpreted as an interaction of facilitation. The three distribution patterns found could be assigned to three different colonization stages: a) the presence of a relative maximum represents the first stage of colonization with isolated clumps of *M. strigosa*; b) an asymptotic pattern indicates uniform, but not saturated, surface coverage and c) surface saturation and constant dependence on distance is characterizated by a linear increase.

Marsilea strigosa generally adopts a vegetative strategy, colonizing from older shoots that also produce sexual nodes. Sexual growth, which is less frequent, seems to be affected by stochastic phenomena that permit the establishment of new clones as seen in pond BV.

The bivariate analysis showed that exclusion does not occur between vegetative and sexual nodes; in fact sexual nodes never occur in isolated clusters, but are interspersed with vegetative nodes. Furthermore, the bivariate analysis indicated aggregation between sexual and vegetative nodes, although this is most likely a result of the connectivity of nodes through runners. Vegetative clumps are common in the first stage of development, but when the number of vegetative nodes reaches a threshold, sexual nodes begin to appear. This should be interpreted as the capacity of a particular network of vegetative nodes to supply enough nutrients to produce a reproductive structure. Spatial point processes are not only able to generate point configurations, but can also reproduce biological phenomena of spatial interactions between points (nodes) with a temporal dimension (patch growth). This method is also effective in identifying various dynamic processes, which can play an important role in the functioning of temporary ponds, and places where these processes are in action. This last point is of particular interest for the concept of an ecosystem-level simulator because the functional units might be considered elementary spatial units that evolve according to specific models of fine-scale dynamics (Pelissier 1998).

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Address of the authors:
David Draper, Universidade de Lisboa, Museu Nacional de História Natural, Jardim Botânico, Rua da Escola Politécnica nº 58, 1200-102 Lisboa, Portugal. E-mail: ddraper@fc.ul.pt.
D. Draper (current address): Dep. Biología Vegetal. ETSI Agrónomos, Universidad Politécnica de Madrid, Av. Complutense s/n. Ciudad Universitaria, 28040 Madrid, Spain. E-mail: david.draper@upm.es.
José María Iriondo, Dpto. Biología Vegetal. E.U.I.T. Agrícola, Universidad Politécnica de Madrid, 28040 Madrid. Spain.