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Disentangling facilitation and seed dispersal from environmental heterogeneity as mechanisms generating associations between savanna plants

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Acacia erioloba; *Grewia flava*; Plant interactions; Spatial association; Wiegand-Moloney *O*-ring statistics.

Nomenclature
Acacia erioloba (E. Mey);
Grewia flava (DC).

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Abstract

Question: How can we disentangle facilitation and seed dispersal from environmental heterogeneity as mechanisms causing spatial associations of plant species?

Location: Semi-arid savanna in the Kimberley Thorn Bushveld, South Africa.

Methods: We developed a two-step protocol for the statistical differentiation of association-promoting mechanisms in plants based on the *Acacia erioloba–Grewia flava* association. Individuals of the savanna shrub *G. flava* and the tree *A. erioloba* were mapped on four study plots. Disentangling the mechanism causing the association of *G. flava* and *A. erioloba* involved tests of three spatial and one non-spatial null model. The spatial null models include homogeneous and heterogeneous Poisson processes for spatial randomness based on the bivariate spatial point patterns of the four plots. With the non-spatial analysis, we determined the relationship between the canopy diameter of *A. erioloba* trees and presence or absence of *G. flava* shrubs in the tree understorey to find whether shrub presence requires a minimum tree canopy diameter.

Results: We first showed a significant positive spatial association of the two species. Thereafter, the non-spatial analysis supported an exclusion of environmental heterogeneity as the sole cause of this positive association. We found a minimum tree size under which no *G. flava* shrubs occurred.

Conclusions: Our two-step analysis showed that it is unlikely that heterogeneous environmental conditions caused the spatial association of *A. erioloba* and *G. flava*. Instead, this association may have been caused by seed dispersal and/or facilitation (e.g. caused by hydraulic lift and/or nitrogen fixation by the host tree).

Introduction

Positive spatial associations between plants of different species, with one species growing beneath or within the other, are frequently observed in arid and semi-arid environments (Munzbergova & Ward 2002; Flores & Jurado 2003). Examples include shrub–cactus associations in the Chihuahuan desert (Yeaton 1978; Yeaton & Manzanares 1986), shrub–grass associations in Patagonia (Soriano et al. 1994), and *Acacia erioloba*–shrub associations in the Kalahari (Milton & Dean 1995; Dean et al. 1999). Such interspecific association can be caused by facilitative interactions, directed seed dispersal or environmental heterogeneities (Phillips & MacMahon 1981; Skarpe 1991; Callaway 1995). In the case of environmental heterogeneity, external factors cause environmental variables to be spatially and/or temporally unevenly distributed. If local environments (e.g. soil nutrient

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concentrations) are favourable to the investigated species, these species will tend to co-occur even in the absence of facilitative interactions (Barot et al. 1999; Maestre et al. 2003). If the association between a mature plant and a seedling is caused by facilitative interactions, the association constitutes a nurse-protégé interaction (Callaway 1995; Flores & Jurado 2003; Malkinson & Jeltsch 2007). A nurse plant has an established canopy beneath which conditions for seed germination and seedling survival are improved, e.g. due to increased water availability, higher soil nutrient concentration or reduced grazing pressure (Flores & Jurado 2003). The protégé plants are seedlings of other species that benefit from the nurse plant but presumably have little effect on their benefactor. However, this commensal relationship may turn into competition once the protégé seedlings grow into established plants (McAuliffe 1988; Flores-Martinez et al. 1994; Miriti 2006). Consequently, the frequency of positive spatial association may decrease with the size of the protégé plants (Miriti 2006).

Positive spatial association between two species cannot simply be taken as support for nurse–protégé interactions or the result of environmental heterogeneity because spatial association may also be caused by directed seed dispersal. Directed seed dispersal (Wenny 2001) results in spatial plant associations similar to those caused by nurse plant effects when wind-dispersed seeds are trapped beneath other plants (Aguiar & Sala 1997; Schurr et al. 2005; Schurr et al. 2008) or when animal-dispersed seeds are preferentially defecated beneath other plant species (Maclean 1970; Milton & Dean 1995; Dean et al. 1999). Directed dispersal can thus generate apparent nurse–protégé patterns such as the typical differences in size of nurse and protégé because the apparent nurse plants are larger than the apparent protégé plants at the time of seed deposition.

How can one distinguish between associations caused by (1) environmental heterogeneity benefiting both species and (2) nurse–protégé patterns caused by facilitative effects on seed germination and seedling survival or apparent nurse–protégé patterns caused by directed seed dispersal? The aim of this study is to develop a protocol for statistical differentiation between these association-promoting mechanisms. We illustrate the use of the protocol by applying it to the savanna tree species *Acacia erioloba* (E. Mey) and the associated fleshy-fruited shrub species *Grewia flava* (DC.). All three mechanisms could potentially explain the positive association of *A. erioloba* and *G. flava*: (1) they occur in spatially heterogeneous environments (Scholes 1990); (2) facilitative mechanisms such as hydraulic lift, stem flow, nutrient enrichment or shade are possible (Caldwell & Richards 1989; Kos & Poschlod 2007); and (3) the fleshy fruits of *G. flava* are transported by animals which may cause directed dispersal to *A. erioloba* sub-canopies (Dean et al. 1999; Schurr 2001). There is good biological knowledge of this system and the mechanisms of association between these two species (Milton & Dean 1995; Dean et al. 1999; Schurr 2001). Following the strategy outlined by Schurr et al. (2004) we predicted *a priori* the deviations from null models that each of these three processes is expected to cause (Table 1), and then tested whether the observed pattern shows these deviations.

Table 1. Null models and statistical tests for association between plant species and for discrimination between different mechanisms underlying an association (i.e. environmental heterogeneity, facilitation and seed dispersal). Steps refer to the protocol proposed in this paper. The last column contains the results of these tests from the example application described in this study. + and − : support and no support for a mechanism, respectively. †: rejection of the null model.

Null model	Statistical analysis	Rejection of the null model is support for . . .		Rejected Example: *G. flava* and *A. erioloba*
		Environmental heterogeneity	Facilitation/seed dispersal	
Step 1				
1. Number of shrubs under tree canopies and in the open are proportional to total canopy area and area in the open	*G*-test	+ (if more shrubs under canopy than open)	+ (if more shrubs under canopy than open)	† (more shrubs under canopy than open)
2. Spatial pattern of the canopies of the shrubs under tree canopies and in the open follows a heterogeneous/ homogeneous Poisson process	*G*-test	+ (if more shrubs under canopy than in the open)	+ (if more shrubs under canopy than in the open)	† (more shrubs under canopy than open in 3/4 plots)
3. Point pattern of the shrub positions with respect to the tree positions follows a heterogeneous/homogeneous Poisson process	Bivariate point pattern analysis	+ (if shrubs and trees are associated)	+ (if shrubs and trees are associated)	† (shrubs and trees are associated)
Step 2				
4. The presence of shrubs beneath the tree sub-canopy is independent of tree size	Randomization procedure	−	+ (there should be a minimum tree size)	† (minimum tree size)

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Methods

The protocol

To identify the underlying mechanism of positive spatial associations between plant species (e.g. a shrub species occurs in the understorey of a tree species), we used a series of three spatial and one non-spatial null models. Null models 1 to 3 relate to step 1 (Table 1) in our protocol, and the aim of these null models was to establish whether there is a positive spatial association between plant species. Here, the exact location of the plants in space matters either implicitly (null models 1 and 2) or explicitly (null model 3). With these spatial null models, we tested whether shrub canopies were Poisson-distributed (= random) over the tree sub-canopy and open areas, and whether the relationship between shrub locations and tree locations was Poisson-distributed. The non-spatial null model (4, Table 1) relates to step 2 of our protocol, separating environmental heterogeneity from facilitative or seed dispersal mechanisms. This null model is not spatial in the sense that it does not depend on the x- and y-co-ordinates of the plants. The presence of shrubs is related to the tree canopy size.

Example: *Acacia erioloba–Grewia flava* association

Study sites

Data were collected in four plots in a semi-arid savanna in the Kimberley Thorn Bushveld (plots 1 and 2) and in a semi-arid *A. erioloba* savanna (plots 3 and 4) in the Northern Cape Province, South Africa, in January and February 2000 (plots 3 and 4) and January 2005 (plots 1 and 2; Table 2). Plots 1 and 2 were situated at Pniel Estates (28°35′S, 24°29′E) 30 km northwest of Kimberley, plot 3 at Dronfield farm (28°37′S, 24°48′E) 10 km north of Kimberley and plot 4 in the Vaalbos National Park (28°28′S, 24°22′E) 60 km northwest of Kimberley.

Mean annual rainfall at the study sites ranges between 370 and 410 mm. At all study sites, rain occurs mainly during the summer months between November and April. On deep sandy soils, the vegetation is dominated by the tree *A. erioloba* and the shrub *G. flava*.

Field methods

All plots were selected with the aim of covering representative stands of the vegetation in the area. A requirement for the spatial analysis (null model 3, Table 1) was that each plot includes at least 30 *A. erioloba* trees. For efficiency reasons, we included two already existing plots (plot 3 and 4, Schurr 2001) that met this requirement. Furthermore, by the use of two study sites (Kimberley Thorn Bushveld, Northern Cape Province) we covered more natural variation of the *A. erioloba–G. flava* association in savannas. Methodological differences between the first two plots compared to plot 3 and 4 were due to differences in logistic constraints at the two sites. In plot 1 and plot 2, all *G. flava* individuals taller than 5 cm and all *A. erioloba* taller than 32 cm were mapped with a GPS device with an accuracy of 1 m, and their height and maximum canopy diameter were measured (Table 2). For single-stemmed individuals, we measured the co-ordinates of the stem base, whereas for multi-stemmed individuals, we estimated the canopy centroid by visual approximation and then determined the co-ordinates thereof. Stems of *G. flava* separated by a distance of up to 10 cm were considered as belonging to the same individual.

Plots 3 and 4 (Table 2) were larger since they were established with the aim of quantifying large-scale neighbourhood effects on the removal and dispersal of *G. flava* seeds (Schurr 2001). In these plots, all *G. flava* individuals taller than 5 cm and all *A. erioloba* taller than 50 cm were mapped, and their height and maximum canopy diameter were measured. The co-ordinates of the stem or, for multi-stemmed plants, the canopy centroid were mapped for *A. erioloba* and *G. flava* in the two plots. The co-ordinates of large trees were obtained from aerial photographs taken in 1993 (South African Surveys and Land Information Bureau, unpubl data) and were then ground-truthed. Positions of isolated smaller plants were calculated by triangulation from the distances to three previously mapped plants, using the INTERPNT computer program (Boose et al. 1998). Co-ordinates of plants located under canopies of other plants were computed from the angle and distance to the stem and canopy centroid, respectively. Stems separated by a distance of

Table 2. Summary of plot information.

Plot	Plot size (m)	Number *G. flava*	Number *A. erioloba*	Density (number *A. erioloba* + *G. flava*/m^2)	Mean canopy diameter of *A. erioloba* with *G. flava* understorey (m)
Plot 1	50 × 100	161	61	0.0400	5.86
Plot 2	75 × 100	66	30	0.0128	8.63
Plot 3	210 × 210	357	140	0.0113	10.83
Plot 4	210 × 210	346	158	0.0114	7.43

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up to 1.5 m were treated as belonging to one individual. The use of 1.5 m compared to 10 cm in plot 1 and 2 is coarser but also more conservative in the sense of not splitting a multi-stemmed individual into several pseudo-individuals. Thus this method should be used if plants tend to be large and multi-stemmed. For all plots, we recorded whether *G. flava* individuals were located under the canopy of another woody species (mainly *A. erioloba*) or in the open.

Step 1: Confirming a positive association between species (*null models* 1–3).

The overall aim of the spatial analyses was to establish if there is a positive spatial association between *A. erioloba* and *G. flava*. The specific aim of the spatially implicit null models 1 and 2 was to find out whether *G. flava* shrubs show preferences for the areas under *A. erioloba* canopies.

Null model 1

In the absence of a spatial association, the number of *G. flava* individuals growing under *A. erioloba* canopies and in the open should be proportional to the area of tree sub-canopy and open habitats. In null model 1 (Table 1) we used a *G*-test (Sokal & Rohlf 1995) to test whether the number of *G. flava* individuals growing under *A. erioloba* canopies and in the open is proportional to the area of *A. erioloba* canopies and open habitats, respectively:

$$G = 2\ln L = 2\left(f_1 \ln\left(\frac{f_1}{\hat{f}_1}\right) + f_2 \ln\left(\frac{f_2}{\hat{f}_2}\right)\right),$$

where f_1 is the observed number of *G. flava* shrubs in the open, $\hat{f}_1$ is the number of *G. flava* shrubs in the open expected if no spatial association occurs, f_2 is the observed number of *G. flava* shrubs beneath the canopy of *A. erioloba* trees, $\hat{f}_2$ is the number of *G. flava* shrubs beneath the tree canopy expected by the occupied area of tree canopies, and L is the likelihood ratio. The calculated *G*-value was compared with a χ^2-distribution (Sokal & Rohlf 1995). A *P*-value <0.01 was taken to indicate that the re-distribution of *G. flava* shrubs was different from the original distribution, i.e. original *G. flava* distribution is not proportional to the area covered by *A. erioloba* trees and the open area.

To determine the impact of the rare occurrence of *G. flava* under the canopy of woody species other than *A. erioloba*, the canopy area of these other plants (e.g. *Ziziphus mucronata*) was first assigned to the open area and, in a second analysis, to the area occupied by *A. erioloba*.

Note that deviation from homogeneity can be problematic because it may lead to apparent or virtual association of species (Wiegand & Moloney 2004; Schiffers et al. 2008). In this case, null model 1 is appropriate only if all spatial heterogeneity experienced by *G. flava* can be characterized by the spatial distribution of *A. erioloba*. We evaluated each plot for its homogeneity using Ripley's *L*-function (Ripley 1976; Besag 1977; for detail see Appendix S1) and found heterogeneity in plot 1, i.e. the intensity λ (mean number of points per area) varies with the location (x, y) within the plot (Stoyan & Stoyan 1994; Wiegand & Moloney 2004).

Null model 2

The problem of heterogeneity in plot 1 can be circumvented through a spatially explicit randomizing of the *G. flava* positions according to a heterogeneous Poisson process (see null model 2, Table 1). Under heterogeneous conditions, to be able to determine if more *G. flava* plants grew under the canopy of *A. erioloba* than in the open, we created a grid matrix (cell side length = 40 cm) representing a plot and treated all plants as objects of finite size (Wiegand et al. 2006). Within this grid matrix, a plant was represented as a point location and a number of cells around the location that corresponded to the recorded canopy extent. The intensity λ in plot 1 varies with location (x, y) within the plot. Therefore, we estimated the intensity $\lambda(x, y)$ in the plot by applying a moving window (Wiegand & Moloney 2004; see also Getzin et al. 2006, 2008; Wiegand et al. 2007) with a bandwidth of 10 m. Then, all *G. flava* individuals were redistributed randomly according to the estimated intensity $\lambda(x, y)$ (heterogeneous Poisson process), whereas *A. erioloba* plants remained fixed (see Wiegand et al. 2006). An area of 10 m is an appropriate estimate of the interaction distance of *A. erioloba* trees (D. Ward, pers. obs.). Consequently, the spatial pattern of plants in the first plot and their deviation from a random distribution can be interpreted up to 10 m only. For plots 2, 3 and 4, in which we found no indication for heterogeneity in the intensity of the point pattern, we applied null model 2 by redistribution of *G. flava* individuals according to a homogeneous Poisson process. After every redistribution, we determined the number of cells for each of the following cell types: cells without *G. flava* or *A. erioloba* (open matrix cells), cells belonging to *G. flava* canopy (*G. flava* cells), cells belonging to *A. erioloba* canopy (*A. erioloba* cells), and cells which were covered by the canopy of both plant species (overlap cells). For all plots, the distribution of *G. flava* canopies in the open and under *A. erioloba* canopies was compared to the distributions in the original plots with *G*-tests (see above; Sokal & Rohlf 1995), where f_1 is the number of *G. flava* cells in the original plot, $\hat{f}_1$ is the number of *G. flava* cells after the random redistribution of the plants, f_2 is the number of overlap cells in the original plot, $\hat{f}_2$ is the number of overlap cells after randomization, and L is the likelihood ratio.

As in null model 1, the calculated G-value was compared with a χ^2 distribution (Sokal & Rohlf 1995). A P-value <0.01 was taken to indicate that the random redistribution of the plants in the grid matrix was significantly different from the original distribution of the plants. For each plot we performed ten simulation runs.

Null model 3

In point pattern analysis, the position of a plant in a plot is represented by a point, and the analysis of the spatial point pattern shows whether the points are distributed randomly. For tests against the spatially explicit null model 3 (Table 1), the spatial distributions of the point patterns of the woody species in the four plots were analysed with the Wiegand-Moloney O-ring statistic (Wiegand & Moloney 2004). The O-ring statistic for a bivariate analysis (point pattern 1: species 1, point pattern 2: species 2) measures the density of points of pattern 2 in a ring of distance h from an arbitrary point of pattern 1. The O-ring statistic is closely related to the pair and mark correlation function (Stoyan & Stoyan 1994) and similar to Ripley's K-statistic (Ripley 1976). However, in contrast to cumulative Ripley's K-statistic, the O-ring statistic uses rings instead of circles (*sensu* Wiegand & Moloney 2004), so that the spatial relation between points can be related to a specific scale. Thus, the O-ring statistic circumvents the memory effect of the cumulative K-function, i.e. a result at a certain distance is not affected by the pattern of smaller distances.

Confidence envelopes for the O-ring statistic were estimated from 999 Monte Carlo simulations of point patterns using a null model. In each simulation, points were relocated according to a specified null model (see Table 1), and the O-ring statistic was calculated for the resulting pattern. We then used the fifth highest and fifth lowest O-ring values at each scale as estimates of the upper and lower confidence interval (representing a significance level of $P=0.01$). The potential error caused by the GPS accuracy of 1 m can be balanced by using a conservative P-value of 0.01 for confidence envelope calculation. Values above the upper confidence envelope indicate significant association between the two point types, whereas values below the lower envelope indicate significant repulsion. Values within the confidence envelope do not differ significantly from the null model.

We calculated the O-ring statistic for a heterogeneous Poisson process for plot 1 and a homogeneous Poisson process for plots 2, 3 and 4 (null model 3, Table 1). The heterogeneous Poisson process of randomization makes it possible to detect second-order effects even if the assumption of most second-order statistics of a homogeneous first-order pattern is violated (as in plot 1). Similar to null model 2, the intensity $\lambda(x, y)$ in plot 1 was estimated using a moving window with a bandwidth of 10 m and every point of the *G. flava* pattern was randomly redistributed according to the estimated intensity to account for the heterogeneity. The *A. erioloba* pattern remained fixed. For plots 2, 3 and 4, we randomly redistributed *G. flava* shrubs, while the *A. erioloba* remained fixed. For null models 2 and 3, we used the software Programita (Wiegand & Moloney 2004).

Step 2: Separating environmental conditions from facilitation and seed dispersal (null model 4)

Null model 4

We determined the relationship between the canopy diameter of *A. erioloba* trees and presence or absence of *G. flava* shrubs in the tree understorey to find out whether shrub presence requires a minimum tree canopy diameter. For each plot, we compared the minimum canopy diameters of trees with shrubs in the sub-canopy to minima emerging from a randomization procedure (null model 4, Table 1). In the randomization procedure, for each shrub observed beneath a tree canopy, we randomly drew a tree canopy diameter from the pool of all observed tree canopy diameters with replacement. We determined the minimum canopy diameter over all shrub–tree associations and repeated the procedure 999 times. The 50th largest value of these iterations gave the upper 95% confidence limit of the minima. If the observed minimum was greater than the upper 95% confidence limit, shrub presence required a minimum tree canopy size that can be distinguished from a random tree canopy allocation process. We also performed the randomization with sampling weighted by tree canopy diameter (Appendix S2). For the non-spatial data analysis, we used the software package R (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org).

Results

Null model 1

In all plots, significantly more *G. flava* plants grew under *A. erioloba* canopies than expected from the G-test distribution of null model 1 (Table 3; see also last column of Table 1 for a summary of all results). This result was independent of whether the canopy area of other woody plant species was lumped with the area of *A. erioloba* (Table 3) or with the open area (result not shown).

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Null model 2

The comparison of the redistribution of *G. flava* canopies with the original position with *G*–tests showed significant differences in plots 2, 3 and 4 for all simulations (null model 2 in Table 1, $G > 785$, $P < 0.01$). Therefore, *G. flava* was more frequent under the canopy of *A. erioloba* than expected under the applied Poisson distribution. In plot 1, we found that three out of ten simulations were not significant (results not shown).

Null model 3

In tests against the heterogeneous and homogeneous Poisson distributions, we found significant associations between *A. erioloba* and *G. flava* at a scale of 3 and 6 m for plot 1 (Fig. 1a). For plot 2, the patterns of *A. erioloba* and *G. flava* were significantly associated at a scale of 2 to 5 m (Fig. 1b). In plot 3, the patterns showed a significant association at 1 to 4 m (Fig. 1c). For plot 4, the two species showed a significant association at 1 and 2 m (Fig. 1d).

The positive association between trees and shrubs is below or similar to the mean canopy diameters of *A. erioloba* trees with *G. flava* in the understorey (Table 2).

Null model 4

In all four plots, we found that *G. flava* only occurred under *A. erioloba* trees above a certain minimum canopy diameter (null model 4). The observed minimum canopy diameters were always greater than the 95% confidence limit produced by randomization (Table 4). When shrub–-tree association was weighted by tree size in the randomization procedure, the observed minimum diameters were still above the upper 95% confidence limit for three out of four plots (Table 4).

Discussion

Positive spatial associations between woody species in savannas can mainly be caused by environmental heterogeneity, facilitation or directed seed dispersal. We provide a protocol to disentangle these association-promoting mechanisms. In the following we will explain our protocol. Thereafter, the application of the protocol is illustrated on the *A. erioloba*–*G. flava* association. In our two-step analysis we investigated the spatial association of the savanna shrub species *G. flava* and the tree *A. erioloba* and disentangled facilitation and directed seed dispersal from environmental heterogeneity. We were able to show that both woody plant species are spatially associated and concluded that environmental heterogeneity is unlikely to cause this association.

Table 3. Non-parametric test statistics and *P*-values for the comparison of *G. flava* numbers under the canopy of *A. erioloba* and in the open with expected proportions (*G*-test; null model 1).

Plot	*G*-test	
	G	*P*-value
1	205.7	< 0.001
2	127.2	< 0.001
3	611.1	< 0.001
4	389.4	< 0.001

The protocol

We suggest proceeding in two steps to investigate positive spatial association of plant species and its underlying mechanism. First, the existence of a positive spatial association has to be confirmed as a basis for further analyses. A significant positive association can be shown by rejection of the null models 1, 2 or 3 (Table 1). Which of these three null models is appropriate for a specific study depends on the system investigated and the available data. Null model 1 is the simplest test for positive associations of species, especially if no information about plant location (x, y) is available or landscape conditions do not allow the use of null model 2 or 3 (e.g. in slope areas). Strictly speaking, null model 1 is valid only in the case of homogeneity. However, because this null model is widely applied, it may also be useful for comparison with other studies, even in the case of moderately heterogeneous intensity. The inference of positive associations is stronger for the spatial null model 2 (Poisson-distributed canopies in the understorey and in the open) and null model 3 (Poisson-distributed point patterns) because they do not depend on the assumption of spatial homogeneity. Both methods can be applied for heterogeneous environments. The strength of null model 2 is the explicit consideration of the size of the plant canopy. Null model 3 reduces plants to point locations, but has the advantage of giving scale-dependent results, thus further ecological knowledge can be acquired about the spatial relation of different species and their underlying mechanism.

If a positive association can be established, the second step is to differentiate the influence of heterogeneous environmental conditions from the other two mechanisms. If the association between the two species is caused by heterogeneous environmental conditions, we should find that both species occur together independent of their sizes, e.g. understorey plants should be found beneath small trees, whereas a facilitative effect of the trees or directed seed dispersal should result in a minimum size of those trees that have shrubs underneath (null model 4). We will illustrate the interpretation procedure in the following by applying it to our case study.

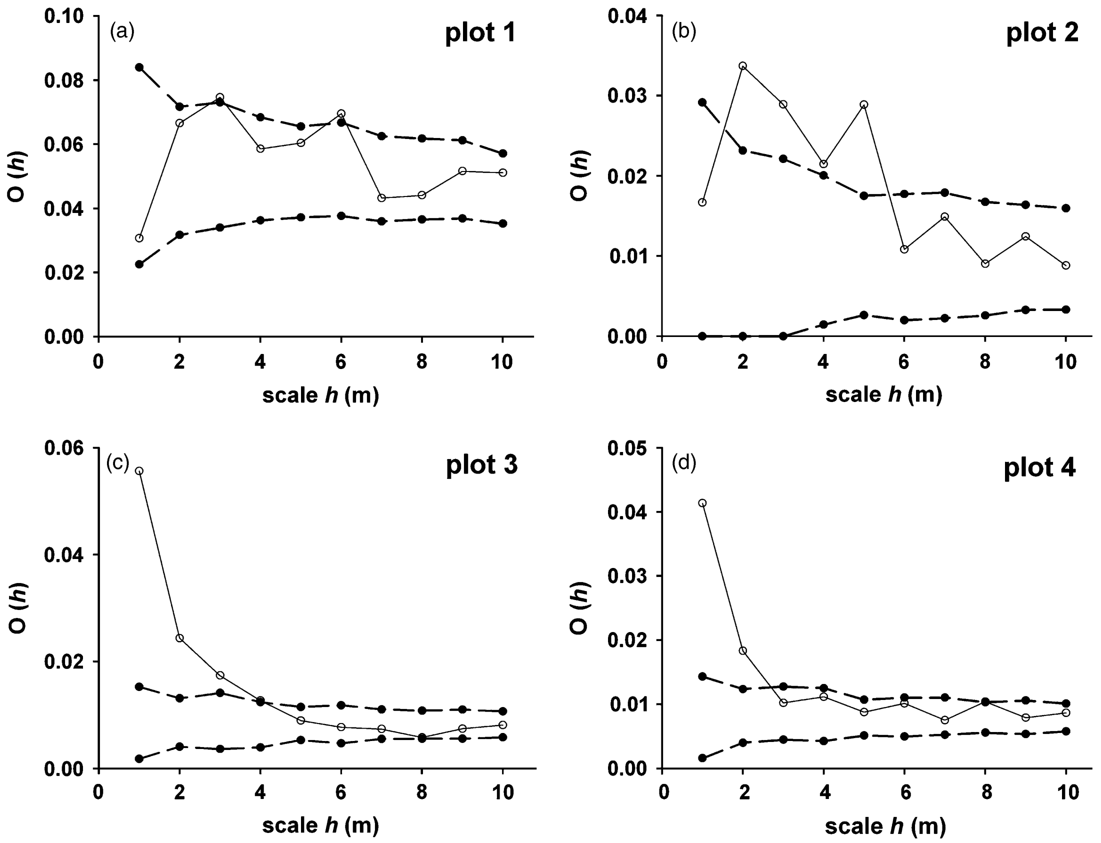

Fig. 1. The *O*-ring statistic (—○—) at different scales *h* with a heterogeneous Poisson process in plot 1 (a), which determines second-order point pattern processes in a heterogeneous plot, and a homogeneous Poisson process as null-model for plot 2 (b), plot 3 (c), and plot 4 (d). For the null model simulations, the intensity $\lambda(x, y)$ in plot 1 was estimated using a moving window with a bandwidth of 10 m and every point of the *G. flava* pattern was randomly redistributed according to the estimated intensity $\lambda(x, y)$ to account for the heterogeneous first-order pattern. The pattern of *A. erioloba* trees remains fixed. For plots 2 to 4, *G. flava* shrubs were redistributed according to a random process (homogeneous Poisson process) while the position of the *A. erioloba* trees remained fixed. Confidence envelopes (– –●– –) were generated from the fifth lowest and fifth highest values out of 999 Monte Carlo simulations of the null model, representing a significance level of approximately $P = 0.01$.

Example: *A. erioloba*–*G. flava* association

Step 1: Confirming a positive association between species (null models 1 to 3).

We showed that there were significantly more *G. flava* individuals under *A. erioloba* canopies than in the open (null model 1). However, null model 1 is valid only in the case of a homogeneous environment, which was not the case in plot 1 (see Appendix S1). Large-scale heterogeneity can be incorporated in null models 2 and 3. We showed that there was more canopy area of *G. flava* under *A. erioloba* than expected from the random redistribution of the plants, considering a heterogeneous intensity in plot 1 and a homogeneous intensity in the other three plots (null model 2). Moreover, the point pattern analyses detected a significant association of the two species on distances below the canopy diameter of *A. erioloba* trees (null model 3). Therefore, we can confirm the positive association between the two species *G. flava* and *A*. erioloba, corroborating similar findings by Milton and Dean (1995), Dean et al. (1999) and Schurr (2001).

Step 2: Separating environmental conditions from facilitation and seed dispersal (null model 4)

Null model 4 provided evidence for the low importance of environmental conditions as an association-promoting mechanism. If environmental conditions caused the association, we would expect to find *G. flava* shrubs under small *A. erioloba* trees by chance alone, whereas facilitation and directed seed dispersal would lead to a minimum size of trees under which shrubs occur, as indicated by our results. A positive effect of an *A. erioloba* plant on *G. flava* germination or survival would only occur if the tree reached a certain canopy size. Also, a tree has to have a certain canopy size to be attractive to animals that transport *G. flava* seeds (Dean et al. 1999), so that *G. flava* seeds accumulate only under larger trees (Schurr 2001).

Table 4. Null model 4, minimum tree canopy diameter with understorey. A randomization procedure (Appendix S2) was used to find whether *G. flava* occurrence needs a minimum tree canopy diameter (facilitation or directed seed dispersal as association-promoting mechanism) or not (environmental heterogeneity as association-promoting mechanism). Observed minimum canopy size of *A. erioloba* trees with *G. flava* in the understorey was compared to the 95% confidence limit calculated by randomization of tree–shrub association (as explained in Appendix S2), not weighted and weighted by tree size.

Plot	Observed minimum canopy diameter of *A. erioloba* trees with *G. flava* understorey (m)	95% confidence limit (unweighted) (m)	95% confidence limit (weighted) (m)
Plot 1	1.02	0.68	1.02
Plot 2	2.30	0.40	2.50
Plot 3	3.79	0.80	2.00
Plot 4	1.05	0.65	1.00

Discussion of our case study application

Our results are consistent with the studies of Milton & Dean (1995) and Leistner (1996) who found that frugivore-dispersed shrubs were more abundant under *A. erioloba* trees than in open vegetation and made up the greatest proportion of the vegetation beneath live *Acacia* trees. We also showed that under heterogeneity, the positive spatial association could be proved (null models 2 and 3). Furthermore, null model 3 showed that the scale of the positive spatial association correlates well with the canopy diameter of *A. erioloba* trees with shrubs in the understorey.

The scale of the spatial association of shrubs and trees varies in plots 1 and 2 compared to plots 3 and 4, which may be caused by vegetation dynamics. A savanna landscape consists of a mosaic of patches that are in different vegetation states (Watt 1947; Scholes & Archer 1997; Wiegand et al. 2005, 2006; Meyer et al. 2007, 2009; Moustakas et al. 2009). The two sets of plots were recorded at different times and in different areas and, thus, it is likely that they are in unequal states of a vegetation dynamic process: trees and shrubs in the four plots are of different age and size, and therefore are exposed differently to processes such as shrub–shrub and shrub–tree competition. Competition between plants increases with the growth of the plants and therefore may lead to a self-thinning process (Wiegand et al. 2008). In plots 1 and 2, a random distribution of trees and shrubs at small distances (Fig. 1) may be caused by increasing tree–shrub competition with growth of *G. flava* shrubs closest to the *A. erioloba* tree, leading to the death of these shrubs. On the other hand, in plots 3 and 4 this competition-based death of shrubs has not yet taken place (perhaps because the shrubs are younger) and, consequently, trees and shrubs are still associated at small distances (Fig. 1).

Dean et al. (1999) explained the positive association of *A. erioloba* and *G. flava* as a two-fold effect of birds and mammals attracted by *A. erioloba*, namely: (1) directed seed dispersal and (2) nutrient enrichment, with the latter indirectly causing facilitation. Dean et al. (1999) suggested that the life span of *G. flava* exceeds the life span of *A. erioloba*, creating a spatially asynchronous cycle. Seeds of *G. flava* arrive under the canopy of *A. erioloba* trees more frequently than in the open due to animal-mediated transport. Seedling establishment is facilitated by ameliorated environmental conditions due to the increased activity of birds and mammals, which are attracted by large trees. The larger the canopy of a host tree (*A. erioloba*), the greater the number of arriving seeds and establishing seedlings in proportion to already established shrubs under the canopy (Dean et al. 1999). Once the *A. erioloba* host tree dies, it leaves a *G. flava* shrub aggregation in the open, where arrival and recruitment of new seedlings is less likely (Dean et al. 1999). This *A. erioloba–G. flava* association will persist and grow until the death of its components. Barnes et al. (1997) and Jeltsch et al. (1996) suggested a life span for *A. erioloba* of 300 and 250 years, respectively, whereas *G. flava* individuals may be able to live for more than 250 years at a high fire frequency and could even exceed 1000 years at a low fire frequency (Schurr 2001).

A potential third step differentiating facilitation from seed dispersal

Finally, if environmental conditions can be excluded as a cause, investigations of seed dispersal and seedling survival can be used to discriminate between directed seed dispersal and facilitation. Therefore, we propose to include a third step into our protocol to distinguish between facilitation and seed dispersal. To decide between the two remaining mechanisms, data on shrub seeds and seedlings and their growth conditions are required. Assuming that soil moisture and nutrients are the most important factors for the spatio-temporal dynamics of savanna shrubs, facilitation can be excluded if micro-environmental analyses of soil moisture and nutrient content show that growth conditions are less conducive under tree canopies than in the open. There is evidence for facilitation if seedlings grow faster or have greater survival rates under tree canopies than in the open. Seedling data with a high spatial and temporal resolution can also be used to exclude seed dispersal if the relative seedling density is greater in the open than under tree canopies. Tests for directed seed dispersal are possible by analysing data on the spatial distribution of shrubs and post-dispersal seed densities with new methods for inverse modelling of fecundity and dispersal in heterogeneous environments (Schurr et al. 2008).

Facilitation and seed dispersal can also occur together. If seed survival data are available, greater survival under tree canopies indicates predominance of the facilitation process. Additional to these observational approaches, experiments can help to distinguish between seed dispersal and facilitation. In seed planting experiments, a greater survival rate under trees than in the open indicates facilitation. Experimental exclusion of seed-dispersing animals and subsequent evaluation of seed germination will reveal the importance of seed dispersal. In the greenhouse, facilitation experiments with artificially enhanced growth conditions may help to determine the role of facilitation. Facilitation experiments with factorial combinations of enhanced moisture and nutrient setups may also contribute to identifying the mechanism by which facilitation operates.

Regarding our *Acacia–Grewia* example, several avian and mammalian dispersers of the fleshy-fruited *G. flava* have been observed in the study area (Schurr 2001), e.g. Acacia pied barbet (*Tricholaema leucomelas*), Glossy starling (*Lamprotornis nitens*), and carnivores such as the Bat-eared fox (*Otocyon megalotis*) and Black-backed jackal (*Canis mesomelas*) who also consume *G. flava* seeds (Skinner & Smithers 1990) and rest under large *A. erioloba* trees (Milton & Dean 1995). In addition, cattle can disperse substantial amounts of *G. flava* seed and shape recruitment patterns in savanna rangelands (Schurr 2001; Tews et al. 2004). Milton & Dean (1995) and Schurr (2001) also found higher concentrations of carbon, nitrogen and phosphorus under *A. erioloba* canopies than in the open area, which is commonly interpreted as evidence for facilitation but could also be caused by nesting bird dispersers and cattle: resting or nesting animals can ameliorate the nutrient availability under tree canopies and contribute to nutrient enrichment (Leistner 1996; Dean et al. 1999). Furthermore, in a *G. flava* germination and establishment experiment by Schurr (2001), emergence probabilities did not differ significantly between four microsites (open, adult *G. flava*, small and large *A. erioloba*). Thus, facilitation of *G. flava* emergence is insufficient to create the observed close association between *A. erioloba* trees and *G. flava* shrubs. Moreover, supplemental watering did not increase emergence of *G. flava*, suggesting that germination is not limited by water availability (Schurr 2001).

Conclusions

We proposed a two-step protocol for identification of the mechanism underlying positive spatial associations between savanna plants based on a series of null models. In our example, we showed that directed seed dispersal and facilitation are both likely mechanisms causing the positive association of *A. erioloba* and *G. flava*. In this case, ecological knowledge of the study system should be used to assess the relative importance of the different null models. Preliminary findings indicated that facilitation might be the less likely mechanism in our case study.

With this protocol, we provide a structured approach to identification of the causes of positive associations that involves several spatial and non-spatial methods. In future investigations, we suggest extending this protocol where possible to include fitness parameters of the species under study, experiments investigating seed dispersal, the spatial analysis of soil conditions and explicit time series data. Bearing this in mind, we believe that our protocol can evolve towards a standardized procedure for disentangling mechanisms underlying any positive spatial association between plant species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Test of homogeneity of shrub and tree spacing.

Appendix S2. Algorithm to create confidence limits by a randomization procedure to determine minimum tree size with understorey.