Spatially explicit analyses unveil density dependence

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Density-dependent processes are fundamental in the understanding of species population dynamics. Whereas the benefits of considering the spatial dimension in population biology are widely acknowledged, the implications of doing so for the statistical detection of spatial density dependence have not been examined. The outcome of traditional tests may therefore differ from those that include ecologically relevant locational information on both the prey species and natural enemy. Here, we explicitly incorporate spatial information on individual counts when testing for density dependence between an insect herbivore and its parasitoids. The spatially explicit approach used identified significant density dependence more frequently and in different instances than traditional methods. The form of density dependence detected also differed between methods. These results demonstrate that the explicit consideration of patch location in density-dependence analyses is likely to significantly alter current understanding of the prevalence and form of spatial density dependence in natural populations.

Keywords: host–parasitoid dynamics; population regulation; spatial analysis by distance indices; spatial density dependence

1. INTRODUCTION

The significance of density dependence as a mechanism regulating species populations has been an active subject of, often heated, debate for at least four decades (Cappuccino 1995; Berryman 2003). Density dependence has until recently largely been viewed from two perspectives, one temporal and the other spatial (Royama 1992; Lundberg *et al.* 2000). Although the time series approach is longest standing, the recognition that the relative spatial position of individuals in populations forms the basis of ecological interactions, in particular those between prey and their natural enemies, led to the adoption of a spatial perspective (Walde & Murdoch 1988). Spatial density dependence, therefore, reflects between-patch variation in the risk of attack to individuals in a prey population, i.e. patch attack risk is dependent on prey density in the patch (Hassell 2000). For example, models of direct, spatially density-dependent attack assume that predators (or parasitoids) will aggregate where prey are aggregated (Walde & Murdoch 1988).

Between-patch variation in attack rate in a prey population is traditionally quantified as the frequency distribution of the number of prey and predators across patches (May 1978; Taylor 1984; Williams *et al.* 2001). However, an overdispersed frequency distribution indicates only that counts associated with sample points vary widely, and provides no information on the physical location of this aggregation in the study area (Perry *et al.* 2002). Therefore, this measure of aggregation is spatially non-explicit, because the spatial positions of patches relative to each other are not considered (Perry 1998). The spatial position of aggregation is, nonetheless, biologically highly relevant

(Roland & Taylor 1997; Perry 1998). For example, a patch with moderate prey abundance may be more heavily attacked if it occurs in the neighbourhood of other patches with moderate to high prey abundance. As a consequence, the outcome of traditional tests for spatial density dependence may differ from those that include ecologically relevant, locational information on both prey abundance and natural enemy attack rates. Despite the obvious importance of determining the significance of using local information for assessing the sign and form of density dependence, this has not yet been done.

One of the most extensive literatures on density dependent relationships, and one that has contributed substantially to our understanding of population dynamics (Statzner *et al.* 2001), is that involving herbivore insects and their parasitoids (Heads & Lawton 1983; Godfray *et al.* 2000). In this literature, the term ‘spatial density dependence’ is associated with a positive relationship between host density and the proportion of parasitized hosts across patches (Lessells 1985; Stiling 1987; Walde & Murdoch 1988). Here, we use a spatially explicit approach to quantify spatial density dependence between an herbivore insect and its parasitoids, and compare the results with traditional, regression model methods. The approach we use is based on spatial analysis by distance indices (SADIE) (Perry & Dixon 2002). It is novel in this context because it quantifies the contribution of each patch to overall aggregation when measuring between-patch variation in both host density and parasitism rate at a site. This contribution, or degree of local aggregation, is then dependent not only on the size of the count associated with a patch, but also on its spatial position and count size relative to neighbouring patches (Perry *et al.* 1999).

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2. METHODS

(a) *Species and sampling*

We quantified the relationship between the pupal density of an African wild silk moth species, *Gonometa postica* Walker (Lepidoptera: Lasiocampidae), and its parasitoids at five sites (populations) within the known outbreak zone of the species in South Africa and Botswana (Veldtman *et al.* 2004). *Gonometa postica* has two generations a year and forms conspicuous silken cocoons on the branches of its host plants. The total number of pupae and number parasitized by an assemblage of three fly and one wasp species was determined on 100 host trees at each site. These data therefore represent a spatial snapshot of *Gonometa* population dynamics, and are therefore equivalent to those used in most spatial density-dependence studies so far. We also consider each site as a single population and make no inferences about the possible metapopulation structure of the system.

These parasitoid species all parasitize the final instar larvae of *G. postica* and emerge from the pupal cocoon (Veldtman *et al.* 2004). Parasitoid species responsible for parasitism are identified from the shape and size of emergence holes left in the cocoon wall (Veldtman *et al.* 2004), and included *Palexorista* sp., *Pimelomyia semitestacea* (Villeneuve) (both Tachinidae), *Brachymeria* sp. (Chalcididae) and an unidentified tachinid (Veldtman *et al.* 2004). Parasitism data were analysed for each species, and collectively ('all species') as a measure of total parasitoid-induced mortality (see also Heads & Lawton 1983; Williams *et al.* 2001).

Gonometa postica populations were examined at Vryburg (26°59' S, 24°40' E, sites 1 and 2, 1.5 km apart) in north-central South Africa, and Gabane (24°37' S, 25°46' E, site 3), Kumukwane (24°38' S, 25°40' E, site 4) and Kopong (24°31' S, 25°48' E, site 5) in southeastern Botswana, spanning a distance of 400 km. Pupal density and parasitism rate were examined in two successive generations at site 3. An initial minimum of 40 cocoons per site was a prerequisite for site selection. The host species used by *G. postica* at the first two sites was *Acacia erioloba* Meyer and at the remainder *Acacia tortillis* Hayne (both Mimosaceae). The position of each tree was measured by using a combination of global positioning system coordinates and categorically assigned percentiles of 1 min. Only trees with at least one pupa were included in density-dependence analyses. We make the assumption, supported by field observations, that final instar larvae do not leave the larval host tree to pupate.

(b) *Analyses*

The results of four, regression-based, methods for the calculation of spatial density dependence were compared with each other and with those of SADIE (software, <http://www.iarc.bbsrc.ac.uk/pie/sadie>; table 1). The overall measure of aggregation and local clustering values (the degree to which local sampling points contribute to the formation of patches and gaps across the sample area) for both host density and parasitism rate were calculated using SADIE (table 1, method 1; figure 1a,b; Perry *et al.* 1999). Spatial association (Perry & Dixon 2002) was then used to compare the local clustering indices of host density and parasitism rate at each shared, spatially referenced point. Significance of associations was determined using critical values for the randomized distribution of overall association (Perry & Dixon 2002). We propose that density-dependent parasitism may be inferred when parasitism rate is spatially associated with host density, and refer to this as spatially associated density dependence (figure 1c). SADIE statistics may be affected by the number and spatial position of patches (Xu & Madden 2003). However, the implications

for multi-patch patterns, as found here, are limited, and the issues raised by Xu & Madden (2003) do not affect the results we report.

A generalized linear model with a binomial error distribution, statistically appropriate for the regression of percentage parasitism data (Collett 1991), was the first of the spatially non-explicit methods used (table 1, method 2). The binomial regression method was also modified to take spatial non-independence of samples into account (table 1, method 3). Significant terms from the third-order polynomial of latitude and longitude records of each tree were first added to the model, followed by host density (Legendre & Legendre 1998). Although the inclusion of locality records in this method does consider the spatial non-independence of counts, it is the spatial structure of parasitism rate that is quantified. However, the spatial structure of host density is also biologically significant in density dependence. By contrast, the spatial association method incorporates spatial structure in both host density and parasitism rate. Although the binomial regression approach has acceptable type I error levels, the type II error risk may be high, especially when underlying density dependence is weak (Trexler *et al.* 1988; Hails & Crawley 1992).

The relationship between parasitism rate and host density has most commonly been quantified by using simple linear regression after arcsine square-root transformation of the proportion of hosts parasitized (table 1, method 4). However, this transformation is generally not adequate to meet least squares assumptions for the proportion of parasitized data (Collett 1991). It also violates assumptions of independence of counts in the presence of spatial autocorrelation and, as a consequence, suffers from inflated type I error rates (Legendre & Legendre 1998).

Finally, a relationship between two variables can be identified as accelerating, decelerating or constant by fitting different regression functions and determining the model with the best fit (table 1, method 5). Linear, exponential, logarithmic and power functions were fitted to each dataset with generalized linear models (assuming a Poisson or negative binomial distribution as appropriate) by using different combinations of the untransformed and transformed dependent (link functions either identity or natural logarithm) and independent variables (untransformed or natural logarithm) (McCullagh & Nelder 1989). The best fitting model was identified by comparing the log-likelihood ratio statistic against the expectation of the χ^2 distribution of competing models (McCullagh & Nelder 1989).

3. RESULTS

Six cases of significant, spatially associated density dependence were detected (table 2, method 1). In all cases the association was positive and in most cases, highly significant. Spatial association identified significant density dependence most frequently. It also found significant density dependence in three cases where none of the other methods did (table 2).

By contrast, using binomial regression all three significant models were negatively density dependent and weak, with pupal density explaining only between 7% and 11% of the deviance in parasitism rate (table 2, method 2). The inclusion of spatial terms in binomial models yielded only a single significant, weak, negatively density-dependent relationship (table 2, method 3). Therefore, although the fit of binomial regression models to parasitism rate was adequate in most cases (deviance per degree of freedom close to unity; McCullagh & Nelder 1989), few relationships were significant, all were negatively density

Table 1. Methods for quantifying spatial density dependence. (LS, least squares; ML, maximum likelihood; exp, exponential; log, logarithmic; SADIE^a.)

	estimation method	Y	treatment of Y	X	treatment of X
1. spatial association ^b	SADIE	proportion of parasitized hosts	rounded integer of proportion multiplied by 10 ^h	number of host individuals	untransformed
2. binomial regression ^{cd}	ML	proportion of parasitized hosts	$\log(y/(1-y))$	number of host individuals	untransformed
3. binomial regression with spatial terms ^e	ML	proportion of parasitized hosts	$\log(y/(1-y))$	(i) number of host individuals; (ii) patch location	(i) untransformed; (ii) significant third order polynomial terms of locality coordinates
4. arcsine square-root ^f	LS	proportion of parasitized hosts	$\arcsin \sqrt{y}$	number of host individuals	untransformed
5. regression function comparison ^g	ML	number of parasitized hosts	linear: y ; exp: y ; log: $\log_e y$; power: $\log_e y$	number of host individuals	linear: $a + bx$; exp: $\exp^x$; log: $\log_e a + bx$; power: x^b

^a Perry *et al.* 1999.
^b This study.
^c Trexler *et al.* 1988.
^d Hails & Crawley 1992.
^e Roland & Taylor 1997.
^f Williams *et al.* 2001.
^g Srivastava & Lawton 1998.
^h The proportion of parasitized pupae was transformed to integers after multiplying with a constant and rounding to the nearest integer so that the standard SADIE method could be used with proportional data (Perry *et al.* 1999).

Figure 1. Interpolated (inverse distance weighted (Perry *et al.* 1999)) spatial clustering and association of moth pupae and parasitism rate by the fly *Pimelomyia semitestacea* (at site 5). (a) Spatial clustering of pupae (overall aggregation statistic $I_a = 0.94$; $p = \text{n.s.}$). (b) Spatial clustering of *P. semitestacea* parasitism rate (overall aggregation statistic $I_a = 1.16$; $p = \text{n.s.}$). In both (a) and (b) areas coded as more than 1.5 denote areas of significant positive (v_i), and less than -1.5 areas of significant negative (v_j) clustering (Perry *et al.* 1999). (c) Spatial association between number of pupae and parasitism rate ($X = 0.453$; $p < 0.001$). Areas coded as more than 0.5 are significantly positively associated ($+X$) at the between-patch scale, whereas those of less than -0.5 are significantly negatively associated ($-X$).

dependent. Two of the cases identified as significantly negative by this method were not identified as significant (either negative or positive) by any other method.

With the widely used arcsine square-root method, five significant, weakly positive relationships between parasitism rate and host density were found (table 2, method 4). Although this method identified weak cases of density dependence, as outlined earlier it does not meet least squares assumptions for proportion parasitized data. Finally, using the regression function comparison approach, relationships were exclusively density independent in those instances where it was possible to discriminate statistically between the four alternative models (table 2, method 5). Regression function comparison was thus the least sensitive method (see also Trexler *et al.* 1988). In summary, there was little agreement between the results of spatial association and those of the four regression-based methods.

4. DISCUSSION

Spatial association appears to offer a superior alternative to traditional methods of quantifying spatial density dependence for several reasons. As evident here, by using explicit locational information for each patch, SADIE has greater power to detect departures from random than other aggregation measures (Perry 1998). Similarly, a measure of spatial matching between the spatially explicit aggregation patterns of two variables is more sensitive to departures from random than measures that ignore the physical location of patches (Winder *et al.* 2001). Furthermore, the method does not violate statistical assumptions of spatial independence (Perry *et al.* 2002), and incorporates

biologically relevant spatial information (Perry 1998) in both host density and parasitism risk.

Using traditional regression-based methods, approximately half of the relationships examined between insect herbivores and their parasitoids so far have been found to be density independent, one-quarter positively density dependent, and the remaining quarter negatively density dependent (Stiling 1987; Walde & Murdoch 1988). Both the infrequency and sign of these density-dependent relationships have been attributed to host and natural enemy characteristics (Heads & Lawton 1983; Lessells 1985; Roland & Taylor 1997; Williams *et al.* 2001), to the scale of the study (Ray & Hastings 1996; Norowi *et al.* 2000), and to the low statistical power of the tests used (Hails & Crawley 1992). Nonetheless, infrequent, weak density dependence is considered the norm under natural conditions (Hails & Crawley 1992; Norowi *et al.* 2000). The results we report here suggest that the method used to quantify spatial density dependence may have been far more important in reaching this conclusion than currently recognized. If the results we report apply more generally, an outcome supported by both biological and statistical arguments, positive spatial density dependence is at least twice as frequent in natural populations than previous studies suggest, and incorrectly identified in up to a third of cases. This has profound implications for our current understanding of population regulation, and may also alter the outcome of spatio-temporal population models.

Nonetheless, regardless of method adopted, the fact that markedly different conclusions on the prevalence, sign and identity of spatial density dependence are reached with alternative methods calls for a re-evaluation of its statistical

Table 2. Relationship between parasitism rate and pupal density.

(Values in bold denote significant density dependence; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Underlined values indicate loss of significance after column-wide step-up false discovery rate correction (at $\alpha = 0.05$) (García 2004). Method 1: overall association (X) (ranging between 1 (perfect association) and -1 (perfect disassociation)). Method 2: percentage deviance explained followed by the sign of the relationship in brackets. Method 3: percentage deviance explained by host density after removing significant spatial terms. Method 4: degrees of freedom associated with the F -statistic are 1, $n-2$ in each case, and the regression coefficient b_{yx} . Method 5: results with different letters in superscript denote significant differences and rank for best fitting curve ($a > b > c$) (exp, exponential; log, logarithmic). The large difference in the percentage deviance explained between methods 5 and methods 2 and 3 is attributable to the expression of parasitized hosts as a proportion in the latter two (see § 2).)

parasitoid/s	1. spatial association	2. binomial regression	3. binomial regression with spatial terms		4. arcsine square-root		5. regression function comparison			
	X	percentage DE	spatial terms	percentage DE host density	F -statistic	b_{yx}	percentage deviance explained (DE)			
							linear	exp	log	power
site 1 ($n = 53$)										
<i>Palearctica</i> sp.	<u>0.310</u>*	2.45	x^2	0.71	0.14	0.008	73.4 ^a	62.5 ^b	60.0 ^c	73.7 ^a
all species	0.207	3.19	x^2, y	0.46	0.00	0.000	82.8 ^a	71.9 ^b	67.4 ^c	82.9 ^a
site 2 ($n = 55$)										
<i>Brachymeria</i> sp.	0.242	0.09	—	0.09	12.47***	0.018	52.3 ^a	32.2 ^c	45.2 ^b	49.6 ^a
<i>P. semitarsata</i>	0.092	0.34	x^2	0.20	0.38	0.005	55.1 ^a	46.6 ^b	43.1 ^c	55.2 ^a
all species	0.253	0.26	—	0.26	2.01	0.012	80.1 ^a	58.9 ^c	66.5 ^b	80.0 ^a
site 3 (generation 1, $n = 60$)										
<i>Brachymeria</i> sp.	0.071	10.46** (—)	x^2	3.32	1.34	0.004	47.0 ^a	48.3 ^a	25.8 ^b	50.5 ^a
<i>P. semitarsata</i>	0.307***	2.85	—	2.85	5.84*	0.007	50.0 ^a	38.0 ^b	33.0 ^b	50.4 ^a
all species	-0.112	11.37*** (—)	x^2, y	2.24	2.55	0.007	66.6 ^a	59.9 ^b	42.9 ^c	68.5 ^a
site 3 (generation 2, $n = 56$)										
<i>Brachymeria</i> sp.	-0.036	0.55	—	0.55	15.00***	0.014	66.2 ^a	43.5 ^c	55.5 ^b	63.5 ^a
<i>P. semitarsata</i>	0.418***	5.26	—	5.26	4.95*	0.008	54.1 ^a	44.9 ^b	37.2 ^b	56.7 ^a
all species	0.307*	7.52* (—)	—	7.52* (—)	9.44***	0.017	81.3 ^a	79.3 ^b	78.3 ^c	82.3 ^a
site 4 ($n = 51$)										
Tachinidae sp.	0.180	4.98	—	4.98	0.37	-0.008	21.8 ^a	22.3 ^a	23.0 ^a	21.9 ^a
<i>P. semitarsata</i>	0.307	1.43	—	1.43	0.00	0.000	14.5 ^a	14.6 ^a	14.9 ^a	14.5 ^a
all species	0.210	3.64	—	3.64	1.08	-0.019	36.1 ^a	32.7 ^a	33.0 ^a	36.2 ^a
site 5 ($n = 38$)										
<i>P. semitarsata</i>	0.453***	0.02	—	0.02	0.67	0.031	17.9 ^a	15.3 ^a	19.2 ^a	17.5 ^a
all species	0.581***	0.04	—	0.04	3.11	0.071	37.9 ^a	24.5 ^b	43.3 ^a	34.4 ^{ab}

definition. While the debate on the consequences of density dependence for host population dynamics continues (Godfray & Hassell 1997; Haak 2002; Berryman 2003), the statistical definition and quantification of density-dependent relationships remain fundamental to the field.

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