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The spatial distribution of larvae of *Culicoides impunctatus* biting midges

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Abstract. The spatial distribution of *Culicoides impunctatus* Goetghebuer (Diptera: Ceratopogonidae) larvae was sampled at a site in western Scotland, and geostatistical analyses were used to quantify spatial dependencies. Nested sampling and analysis indicated that most of the spatial variance occurred within distances of 160 640 cm, levelling off at distances >640 cm. Semivariograms for transformed data from three 100 m × 100 m grids showed similar, isotropic patterns for larval counts, soil organic content, soil water content and the distribution of *Juncus* spp. rushes, with the variance increasing with separation distance. High levels of significance were associated with power models used to describe the semivariograms, which was indicative of the absence of a plateau (or 'sill') in the respective data. Correlation analysis of transformed data revealed significantly positive relationships between larval counts and soil pH, soil percentage organic content, soil percentage water content and also the distribution of *Sphagnum* spp., *Juncus* spp. and *Myrica gale*. There were also significantly negative relationships between larval counts and the distribution of *Pteridium aquilinum* and all mosses other than *Sphagnum* spp. The results suggest a far more structured and predictable pattern of *C. impunctatus* larval sites than previous studies and are discussed in relation to their application in localized *Culicoides* control and to studies of the mechanisms determining the spatial distribution of *C. impunctatus* larvae.

Key words. *Culicoides impunctatus*, larval aggregations, geostatistics, Scotland.

Introduction

Culicoides impunctatus Goetghebuer is the commonest of thirty seven species of *Culicoides* biting midge in Scotland (Boorman, 1986). This species is responsible for the majority of biting attacks on humans during the summer months in many Highland and West Coast areas of Scotland and has a significant impact on tourism and outdoor industry (Hendry & Godwin, 1988; Blackwell, 1997). In addition to being a biting pest, this species is of potential veterinary importance, given its ability to act as a vector for two important viral pathogens of livestock: epizootic haemorrhagic disease virus of deer (Boorman & Gibbs, 1973) and blue tongue virus of ruminants (Jennings & Mellor, 1988).

The success of *C. impunctatus* in Scotland has been attributed partly to the fact that the females are autogenous, not requiring a blood meal to lay their first batch of eggs, and partly to the vast areas of potential habitat for this species (Boorman & Goddard, 1970; Blackwell *et al*., 1992). The preferred blood meal hosts following the first batch of eggs are cattle and deer, with humans being fed upon only opportunistically (Blackwell *et al*., 1994a, 1995). *Culicoides impunctatus* is on the wing from approximately late May to late September, with two generations per season (Blackwell *et al*., 1992), overwintering as final, fourth stage larvae in the damp, acid soil of their natural habitat.

The numbers of *C. impunctatus* larvae vary in both time and space, which is of interest in the study of their population dynamics and also of practical importance in control measures aimed at the larval stages. Recently, transect surveys have been used to investigate both the horizontal and vertical distribution of *C. impunctatus*. Horizontally, regression analysis identified significant relationships between larval numbers and a soil 'wetness index' and also the distribution of *Juncus acutiflorus/J. articulatus* (Juncaceae) (Blackwell *et al*., 1994b), and vertically 50% of larvae were found within the first 2 cm of the soil, with

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little movement over a 24 h period (Blackwell & King, 1997). These studies added to an earlier descriptive study of the moorland vegetation associated with *C. impunctatus* breeding grounds (Kettle, 1961). However, the efficiency of larval collection remains low, which may be related to the fact that in these previous studies no attention was paid to any spatial dependencies within either the larval distributions or the major environmental variables. Such information, particularly con cerning larval aggregations, is imperative if sampling strategies are to be optimized and if accurate estimates are to be made of midge populations and used, for example, to predict areas of potentially high midge activity, to identify key areas to target in control programmes or even as indicators of soil quality.

A variety of mathematical techniques have been employed to determine the spatial distribution of other soil dwelling organisms. In particular, geostatistical techniques, which quantify spatial dependencies, have been applied to data for a variety of soil nematodes (Robertson & Freckman, 1995; Marshall *et al*., 1998) and for subterranean termites, *Reticuli termes tibialis* (Crist, 1998). They have also been applied to the spatial patterns of species richness for aerial insects, including *Lymantria dispar* (Lepidoptera: Lymantridae) (Sharov *et al*., 1996), *Abax parallelepipedus* (Coeleoptera: Carabidae) (Fran ceshini *et al*., 1997‘) and various tiger beetle species (Coelop tera: Cicindelidae) (Carroll & Pearson, 1998). These techniques, which in addition have been used extensively to describe variations in soil properties (Bourgault *et al*., 1997; Crawford & Hergert, 1997; Bardossy & Lehmann, 1998; Gorres *et al*., 1998), particularly the spatial structure of soil pollutants (Kuzel *et al*., 1994; Einax & Soldt, 1995), have the advantage that they allow the intensities of a given organism/property to be mapped, identifying areas of high and low density in a given area, which can be compared with similar maps for other factors with which they may have some association. They are a particularly useful tool with soil living organisms, which often exhibit complex spatial patterns at various scales. Standard aggregation indices or probability distribution analyses do not take into account the location of the sampling sites and they provide no information at scales outside of the sampling unit size. Geostatistical analysis, however, analyses the spatial pattern of a variable at scales ranging from the minimum to the largest intersample distance (Rossi *et al*., 1995).

The aim of this study was to use geostatistical techniques to investigate the distribution of *C. impunctatus* larvae and to study the spatial patterns of the associated soil properties and the main plant groups in the larval habitat, and to confirm any similarities in spatial pattern using conventional statistical analysis.

Materials and Methods

Study area

The study was based on the Ormsary Estate, near Lochgilp head, Argyllshire, National Grid Reference 743 724 (56°N, 5°W, 50 150 m a.s.l.), which has been used for two previous studies of *C. impunctatus* larval distribution (Blackwell *et al*., 1994b; Blackwell & King, 1997). Three sites were chosen within this area.

Site 1 was a mixed area of open grass and sparse birch (*Betula pubescens*) woodland, surrounded by a bank of bracken, *Pteridium aquilinum* (Pteridophyta). Site 2 was another sparse *B. pubescens* wood, interspersed with damp, open areas dominated by mosses including *Sphagnum* spp. (Bryophyta) (primarily *S. papillosum*), grasses (mainly large tussocks of *Molinia caerulea* (Gramineae)) and Sweet gale, *Myrica gale* (Myricaceae). Site 3 corresponded to site 1 of Blackwell *et al*. (1994b); also an open *B. pubescens* woodland, interspersed with stands of *P. aquilinum* and damp areas dominated by mosses and *Juncus* spp. rushes (primarily mixed stands of *J. acutiflorus* and *J. articulatus* (Juncaceae)). All three sites conformed largely to the description of *Betula pubescens Molinia caerulea* (Gramineae) (*Sphagnum* spp. (Bryophyta) subcommunity) (W4 of the National Vegetation Classification (NVC)) (Rodwell, 1991).

Sampling and larval counting

Sampling of the overwintering *C. impunctatus* larval population was carried out in October and November 1996. At each sampling point, one (nested survey) or two (grid survey) soil cores were removed, using an auger to give cores of standard size; 10 cm diameter, cut to 8 cm depth (the limit for reliable recovery of *C. impunctatus* larvae (Blackwell & King, 1997)). Each core held $\approx 628\,cm^3$ of fresh soil. Individual cores were kept separate and stored at 4 5°C until use. One core from each sampling point was used for larval counts; cores were dried for 72 h in Tullgren funnels, after which extracted *Culicoides* spp. larvae were identified using a standard key (Kettle & Lawson, 1952). The second core from each sampling point of the grid survey was used for soil analysis. In addition, percentage vegetation cover was noted at each sampling point of the grid survey, using the following categories: (I) grasses and sedges (mainly *Molinia caerulea* tussocks, with smaller amounts of *Deschampsia caespitosa, Holcus lanatus* and *Festuca ovina* (Gramineae), with the main sedges being *Carex echinata* and *Carex nigra* (Cyperaceae)); (II) *Sphagnum* spp. (mainly *S. papillosum*, with smaller amounts of *S. auriculatum* var. *auriculatum* and *S. palustre* (Bryophyta)); (III) other mosses (*Polytrichum commune*, *Rhytidiadelphus loreus*, *Rhytidiadelphus squarrosus*, *Pseudo scleropodium purum*, *Mnium hornum*, *Plagiothecium undula tum* (Bryophyta)); (IV) herbaceous plants (not identified to species due to sampling being carried out outside of the flowering season); (V) *Pteridium aquilinum*; (VI) rushes (*Juncus acutiflorus*, *J. articulatus* and *J. effusus* (Juncaceae)); (VII) *Myrica gale* (Myricacaea); and (VIII) heather (*Calluna vulgaris*, *Erica tetralix* (Ericaceae)).

Nested survey

Variation in *C. impunctatus* larval numbers could occur over a wide range of scales, possibly over several orders of

magnitude of distance. The total variation in a given area will be the sum of the contributions from different parts of the range, some of which will be large and some of which will be small. In order to determine the spatial scale over which *C. impunctatus* larval variation occurred, a nested survey was undertaken (Oliver & Webster, 1986; Webster & Boag, 1992). An initial sampling point ('node') was chosen within site 1 (above) and distances were selected from this point in a geometric series (i.e. 10, 20, 40 cm, increasing to 2560 cm, with ten nodal points). The angle between each point and the next was chosen randomly, using the eight points of the compass. There were four replicates of this sampling series within site 1, with the initial sampling points selected using previous experience of areas where there was a high probability of the presence of *C. impunctatus* larvae. This resulted in there being several pairs of points separated by the same distances, and because the sampling points were nested each occurred only once in each class of distance in a hierarchy, and hence contributed information to the variance at every distance.

Grid survey

Grid surveys were carried out at all three sites. Grids (100×100 m) were marked out at 10 m intervals (identified as a reliable distance over which significant variation in larval numbers occurred from the nested survey). This gave 121 points per grid, which were sampled as above, with associated vegetation descriptions.

Soil analysis

Soil samples for analysis of pH, water content and organic content (loss on ignition (LOI)) were passed through a 2 mm sieve and 10 g subsamples ($n = 5$ per soil sample) were dried to a constant weight at 60°C for 48 h. The percentage water content of each subsample was determined from the dry weight and the pH of each oven dried subsample was also measured (0.5 g + 1 ml distilled water). The percentage LOI (8 h at 450°C) was determined from the remaining oven dried soil.

Statistical analysis and mapping

Data sets for percentage vegetation cover, LOI and water content were transformed using arcsine ($\text{asin}\sqrt{}$) and larval densities were normalized using a $\log(n+1)$ transformation. Geostatistical analysis of both nested and grid survey data were carried out using GSLIB software (Deutsch & Journel, 1992). Spatial variability was measured by calculating semivariograms (γ) for larval counts, soil properties and vegetation groups. The semivariogram represents the *lag* or separation distance between pairs of samples (1, 2, etc. grid squares). Statistically, the semivariogram represents the semivariance (variance/2) between pairs of samples (i.e. a measure of the squared differences in counts between paired samples). For the grid survey, semivariance was measured in two orthogonal directions along the main axes of the grid (i.e. North and East directions), according to the following formula:

$$\gamma(h) = 1/(2N(h))\sum_{i=1}^{N(h)}(x_i - y_i)^2 \qquad (1)$$

where $N(h)$ is the total number of pairs, separated by distance h, and x_i and y_i are the values at either end (i.e. south and north or west and east or ends, respectively) of the ith sample pair. For the grid survey data, the spatial dependency of the observed semivariance was described using a power model (Deutsch & Journel, 1992).

Observed similarities in the patterns of spatial variation between measured variables were further investigated by calculating Pearson's correlation coefficients (MINITAB (1994) for Windows, Release 10.1).

Results

Nested survey

Larval numbers generally decreased from the initial sampling point (Table 1), resulting in an increase in the semivariance between mean larval counts at increasing intersample distances. The largest increases in semivariance occurred at an interval of 80 cm (i.e. between sampling distances of 80 cm and 160 cm); the high semivariance at 160 cm was due to a count of zero in one replicate. There was also evidence of a plateau ('sill') at intervals of 640 cm and 1280 cm, suggesting that the limit of spatial dependence had been reached (Fig. 1). From this data, an intersample distance of 10 m was selected for the grid survey.

Grid survey

Distinctive patterns of spatial variation were recorded for *C. impunctatus* larval counts, percentage soil water content and percentage soil organic content (LOI) at all three survey sites, and also for the distribution of *Juncus* spp. rushes at site 3 (Figs 2 4). The maximum numbers of larvae per soil core varied between 0 and 27 (site 1), 44 (site 2) and 30 (site 3).

For site 1, distinctive aggregations of larval numbers occurred in the south west of the survey grid, dispersing westwards with smaller patches of high density (Fig. 2a). Similarly, aggregations of high soil LOI occurred in the south west corner of the grid, with further patches of high LOI extending 50 m northwards and at the extreme easterly edge of the grid (Fig. 2b). Larval aggregations and high soil organic content were matched by areas of high soil water content in the south west corner, spreading in both northerly and easterly directions (Fig. 2c). *Juncus* spp. rushes were recorded from only five of the sampling points and hence not mapped in this way.

A relatively large aggregation of larvae occurred in the north east corner of site two, with a smaller aggregation in the

Table 1. Mean (± SE) *C. impunctatus* larval numbers recovered from soil cores sampled in the nested survey (n 4).

Lag distance (cm)	0	10	20	40	80	160	320	640	1280	2560
Larvae	9.5 ± 3.8	13.0 ± 4.3	10.8 ± 4.2	9.8 ± 3.8	7.0 ± 3.1	8.2 ± 1.9	3.8 ± 3.4	5.5 ± 2.2	3.5 ± 3.5	0.1 ± 0.2

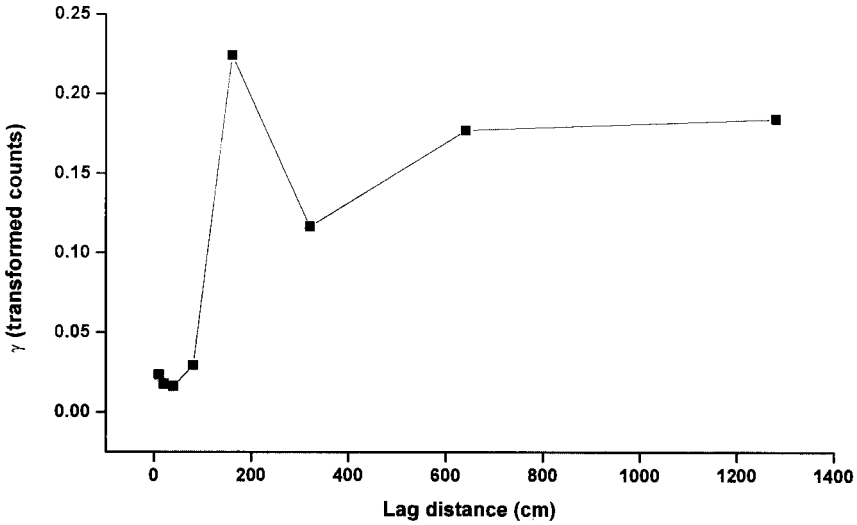

Fig. 1. Semivariograms (γ) of transformed (log(n + 1)) larval counts against lag distance (i.e. separation distance).

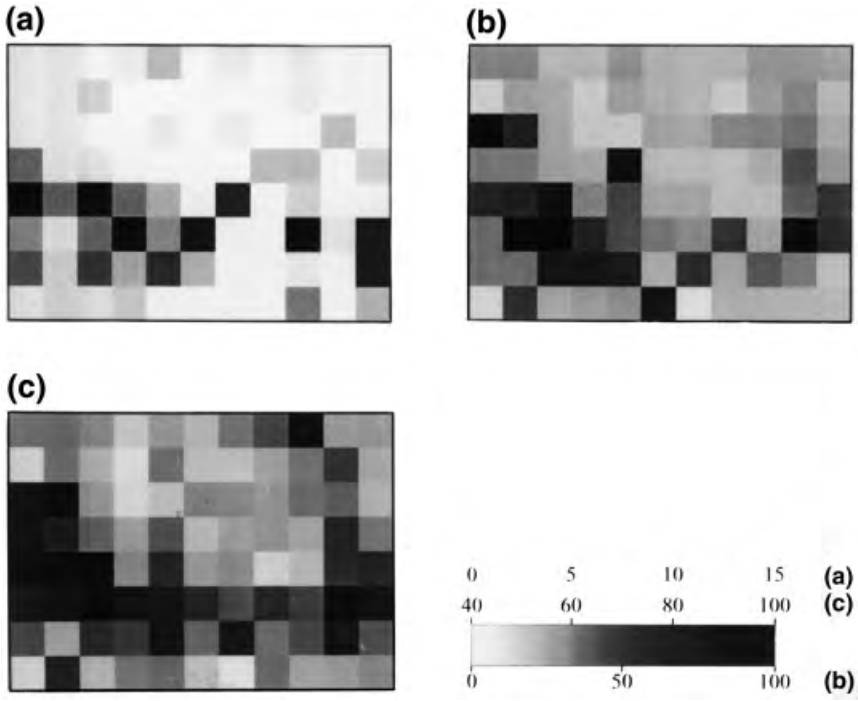

Fig. 2. Spatial density distribution of (a) *C. impunctatus* larvae, (b) soil organic content (% LOI) and (c) soil percentage water content at site 1 (100 × 100 m). Untransformed, raw data used for all variables.

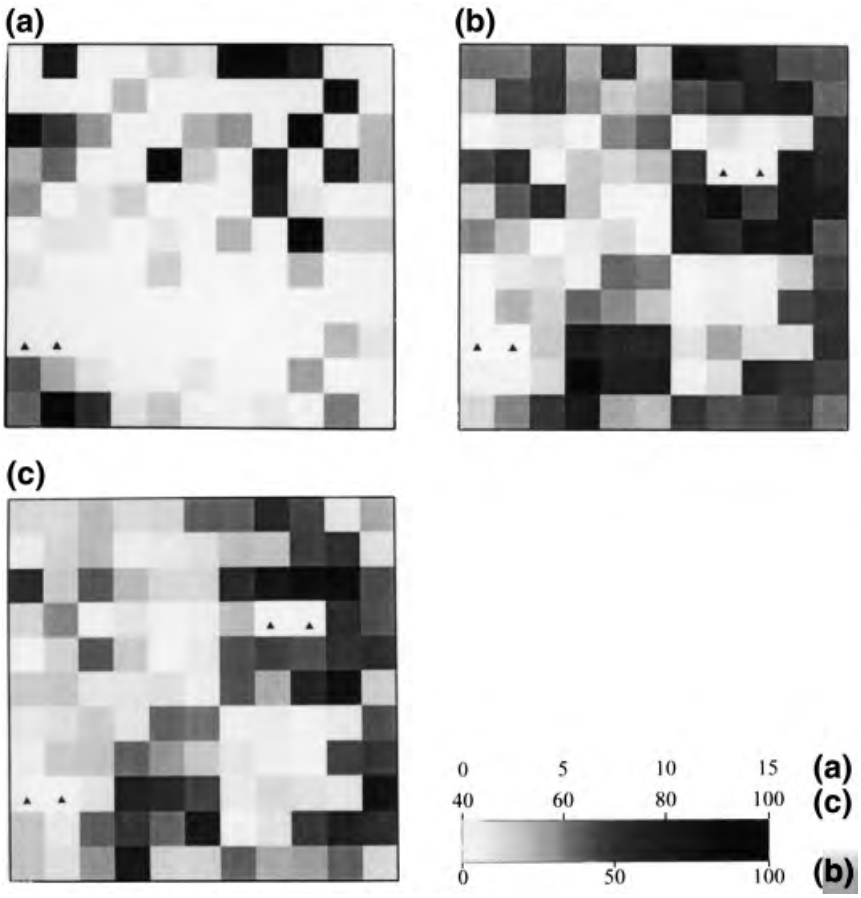

Fig. 3. Spatial density distribution of (a) *C. impunctatus* larvae, (b) soil organic content (% LOI) and (c) soil percentage water content at site 2 (100×100 m). Untransformed, raw data used for all variables. ▲ missing value.

south west corner (Fig. 3a). Areas of low larval density were matched by similar areas of low values for both soil LOI and water content, with aggregations of higher values for these properties at the north east, south east and south west corners of the respective grids (Fig. 3b, c). There were no *Juncus* spp. rushes recorded at the sampling points within site two.

Culicoides impunctatus larvae were strongly aggregated in the north east and south west corners of site 3, with a trough of low density running from north west to south east (Fig. 4a), which was also present in the distribution of values for soil LOI and water content, with aggregations of high values in the north east and south west corners (Fig. 4b, c). Aggregations of *Juncus* spp. rushes occurred in the south west corner of the grid (Fig. 4d), corresponding with the larval aggregations.

The semivariograms of the transformed values showed similar trends for all three sites, although the patterns were most obvious for site 3 and hence have been used to describe how the variance in the measured properties changed over distance (Fig. 5). For larval counts and for the two soil properties for which patterns of spatial variation were identified through mapping, the semivariance increased with lag distance, giving positive slopes. Generally, the effects were isotropic, with the variances in both northerly and easterly directions increasing by similar amounts. The steeper increase in variance in the northerly direction for larval counts mirrors the more pronounced change in larval numbers in a north south direction seen in Fig. 4(a). Changes in variance for the distribution of *Juncus* spp. rushes was less regular, mirroring the patchy distribution of this plant group within the grid (Fig. 4d).

None of the semivariograms for larval counts, soil percentage organic content or soil percentage water content at site 3 attained a complete plateau or 'sill' (i.e. a complete levelling off at greater distances) and therefore to describe the scale dependency of the observed semivariance a power model to the data '(Deutsche & Journel, 1992') $\gamma(h) = ch^a$ with c and a being estimated parameters, derived by taking logarithms of the lag distance (h) and the semivariogram ($\gamma(h)$) and fitting a linear regression line $\log(\gamma(h)) = \log(c) + a \log(h)$.

Power models were fitted to the semivariograms of transformed values in both northerly and easterly directions for larval counts, soil percentage LOI and soil percentage water content. In addition, power models were also fitted to the raw data for larval counts, allowing a physical interpretation of the fitted model (Marshall *et al.*, 1998). The regression relationships were all statistically significant for both northerly

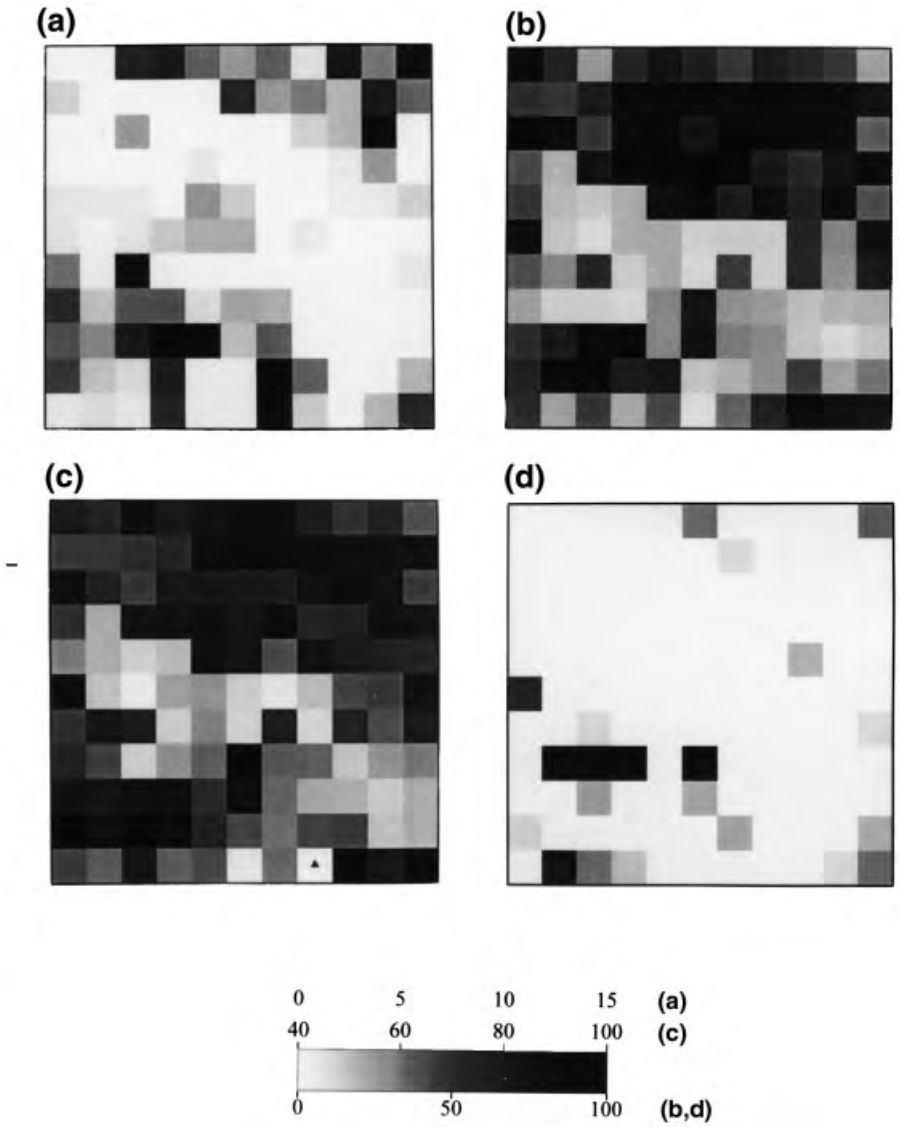

Fig. 4. Spatial density distribution of (a) *C. impunctatus* larvae, (b) soil organic content (% LOI), (c) soil percentage water content and (d) *Juncus* spp. at site three (100 × 100 m). Untransformed, raw data used for all variables. ▲ missing value.

and easterly directions (Table 2). The semivariogram data for the distribution of *Juncus* spp. were unsuitable for further analysis.

Correlation analysis revealed statistically significant, posi tive relationships between the numbers of *C. impunctatus* larvae and soil pH at all three sites, although the range of soil pH values was limited (site 1 pH 3.1 5.0; site 2 pH 3.3 6.5; site 3 pH 1.8 5.5). There were also statistically significant, positive relationships between larval numbers and soil LOI at sites 1 and 2, and between larval numbers and soil water content at sites 1 and 3. There were no clear trends in the relationships between soil pH and either soil LOI or water content, whereas there were highly significant relationships between soil LOI and soil water content at all three sites (Table 3).

With the combined vegetation data for all three sites, statistically significant, positive correlations existed between the numbers of *C. impunctatus* larvae and the proportions of *Sphagnum* spp., *Juncus* spp. and *Myrica gale*, with the strongest relationship being with *Juncus* spp. rushes (although these occurred only in the samples taken at sites 1 and 3). Larval numbers were also negatively correlated with the distribution of *Pteridium aquilinum*. Not all of these relation ships, however, held for the single site data, which also included indications of other relationships, e.g. at sites 1 and 3 there were statistically significant, negative correlations between the numbers of *C. impunctatus* larvae and the distribution of 'other mosses' (i.e. excluding *Sphagnum* spp.) (Table 4).

Table 2. Fitted power models (log(γ(*h*)) log(*c*) + *a* log(*h*)). γ(*h*), semivariogram of the transformed data for larval counts, soil percentage LOI and soil percentage water content, and the raw data for larval counts at grid survey site three; *h*, lag distance (m); log(*c*) and *a*, estimated parameters of the model (logarithm of the semivariance extrapolated to zero lag and the exponent of the Power Law, respectively); figures in parentheses, standard deviations for each estimate; R^2, adjusted percentage variation accounted for with the associated probability value (*P*); N, northerly direction; E, easterly direction.

Variable		*a*	log(*c*)	R^2 (%)	*P*
C. impuncatus larvae	N	0.55 (0.03)	1.57 (0.05)	98.1	<0.001
(transformed data)	E	0.23 (0.05)	1.16 (0.07)	80.2	<0.01
Soil percentage LOI	N	0. 36 (0.05)	1.43 (0.08)	89.7	<0.01
(transformed data)	E	0.48 (0.04)	1.84 (0.06)	96.6	<0.001
Soil percentage water	N	0. 28 (0.03)	1.84 (0.11)	70.5	<0.02
(transformed data)	E	0.58 (0.04)	2.42 (0.07)	96.5	<0.001
C. impuncatus larvae	N	0. 54 (0.04)	0.57 (0.06)	97.2	<0.001
(raw data)	E	0.18 (0.02)	1.09 (0.03)	94.6	0.001

Table 3. Correlation analysis for transformed (log(*n* + 1)) larval counts and soil properties (pH, transformed (asin)% LOI and transformed (asin)% water content) in grid survey samples for site 1 (*n* 88), site 2 (*n* 121), site 3 (*n* 121) and the combined data for the three sites (*n* 330). Pearson's correlation coefficients (*r*) shown with corresponding significance level (*P*). Note: lower sample size for site 1 due to missing samples.

	No. larvae and pH	No. larvae and % LOI	No. larvae and % water	pH and % LOI	pH and % water content	% LOI and % water
Site 1	*r* 0.286	*r* 0.203	*r* 0.386	*r* 0.041	*r* 0.144	*r* 0.716
	P < 0.01	*P* < 0.05	*P* < 0.001	NS	NS	*P* < 0.001
Site 2	*r* 0.230	*r* 0.204	*r* 0.148	*r* 0.189	*r* 0.264	*r* 0.693
	P < 0.05	*P* < 0.05	NS	NS	*P* < 0.01	*P* < 0.001
Site 3	*r* 0.352	*r* 0.056	*r* 0.232	*r* 0.204	*r* 0.004	*r* 0.730
	P < 0.001	NS	*P* < 0.05	*P* < 0.05	NS	*P* < 0.001
Sites 1 3	*r* 0.241	*r* 0.159	*r* 0.234	*r* 0.118	*r* 0.050	*r* 0.730
	P < 0.001	*P* < 0.01	*P* < 0.001	*P* < 0.05	NS	*P* < 0.001

NS, not significant at 5% level.

Table 4. Correlation analysis for transformed (log(*n* + 1)) larval counts and vegetation categories (I, grasses and sedges; II, *Sphagnum* spp.; III, other mosses; IV, herbaceous plants; V, *Pteridium aquilinum*; VI, *Juncus* spp.; VII, *Myrica gale*; VIII, *Calluna vulgaris*/*Erica tetralix*) in grid survey samples for site 1 (*n* 88), site 2 (*n* 121), site 3 (*n* 121) and combined data for the three sites (*n* 330). Pearson's correlation coefficients (*r*) shown with correpsonding significance level (*P*). , vegetation group absent. Note: lower sample size for site 1 due to missing samples.

	I	II	III	IV	V	VI	VII	VIII
Site 1	*r* 0.023	*r* 0.242	*r* 0.253	*r* 0.024	*r* 0.233	*r* 0.369	*r* 0.163	
	NS	*P* < 0.05	*P* < 0.05	NS	*P* < 0.05	*P* < 0.01	NS	
Site 2	*r* 0.04	*r* 0.084	*r* 0.138		*r* 0.090		*r* 0.084	*r* 0.087
	NS	NS	NS		NS		NS	NS
Site 3	*r* 0.014	*r* 0.190	*r* 0.242	*r* 0.125	*r* 0.134	*r* 0.262	*r* 0.254	*r* 0.108
	NS	*P* < 0.05	*P* < 0.05	NS	NS	*P* < 0.01	*P* < 0.01	NS
Sites 1 3	*r* 0.051	*r* 0.159	*r* 0.097	*r* 0.054	*r* 0.154	*r* 0.213	*r* 0.144	*r* 0.003
	NS	*P* < 0.05	NS	NS	*P* < 0.01	*P* < 0.001	*P* < 0.05	NS

NS, not significant at 5% level.

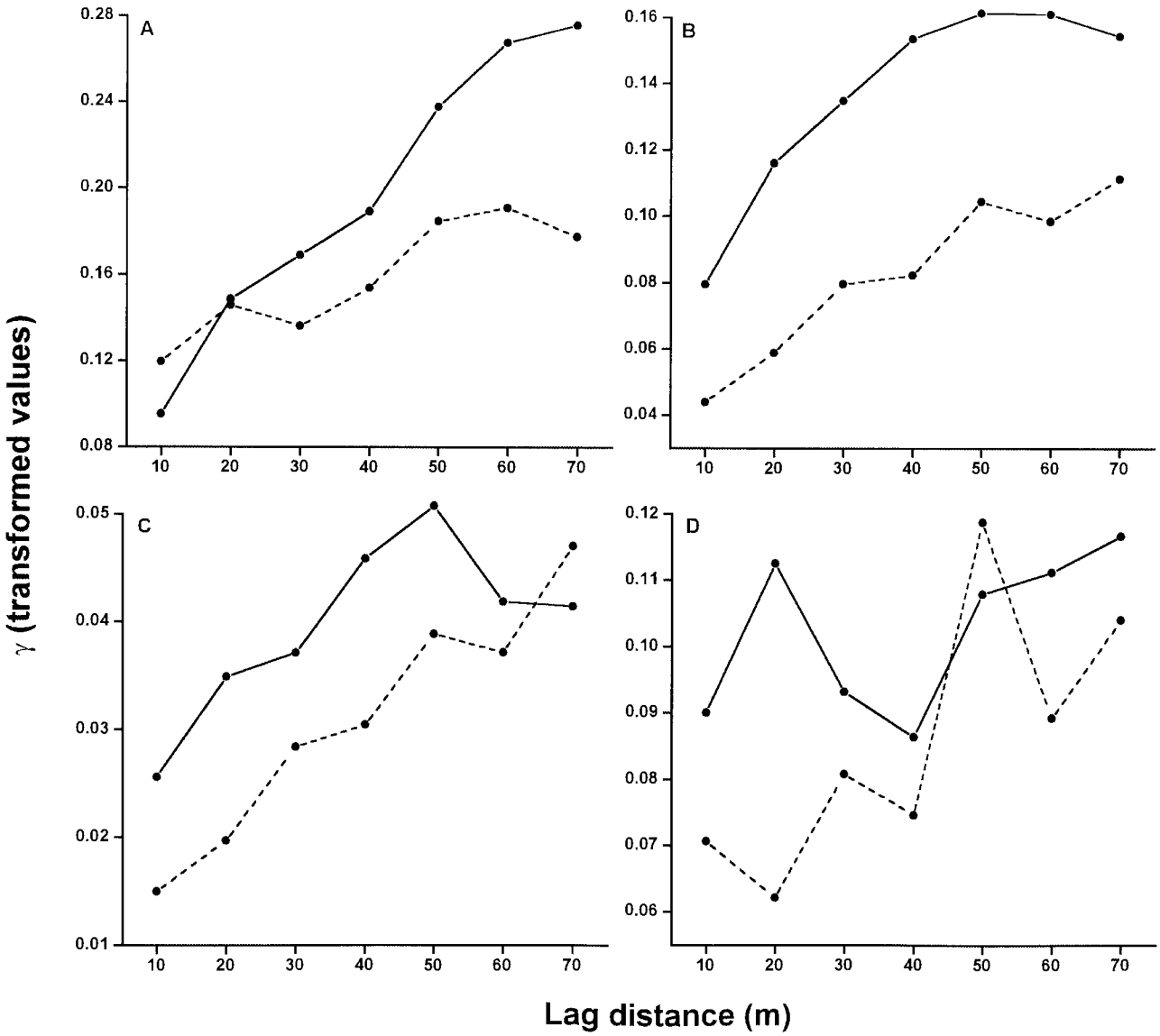

Fig. 5. Semivariograms (γ) of (a) transformed (log($n+1$)) larval counts, (b) transformed (asin) soil percentage organic content (loss on ignition (LOI)), (c) transformed (asin) soil percentage water content and (d) transformed (asin)% *Juncus* spp. cover in grid survey samples for site 3 (n 121). γ shown for two orthogonal directions, north (solid line) and east (dashed line).

Discussion

This is the first study to be carried out of the spatial distribution of *Culicoides* spp. larvae and it has made a significant contribution to the current information on the distribution of the dominant species in Scotland, *C. impunctatus*. The use of a nested sampling scheme (Webster & Boag, 1992) has provided detailed information on the spatial distribution of *C. impuncta tus* over a range of distances, allowing estimates of their spatial scale, which has then been confirmed by more intense grid surveys. The chosen grid size (100×100 m, with a 10 m intersample distance) allowed accurate maps of the spatial density distribution of *C. impunctatus* larvae to be constructed and the spatial patterns within these were then described with semivariograms. The distribution of larvae appeared to be isotropic, with variance increasing with lag distance. Sill variances were not reached, suggesting that the limits of spatial dependence had not been reached and thus indicating that factors influencing the distribution of *C. impunctatus* larvae were still changing at distances greater than those investigated by the present sampling strategy. This was confirmed by a statistically significant fit of a power model (Deutsch & Journel, 1992) to the transformed data for larval counts. A power model was also fitted to the raw data for larval counts, in the same way

as Marshall *et al*. (1998), who discussed the use of the power model in terms of a naturally occurring scaling model, 'fractional Brownian motion', deriving a value H (= a/2 from the power model (Table 2)), with a value of $H \cong 0.5$ representing random 'Brownian motion'. From the present data, values of H for larval spatial distribution can be calculated of 0.27 and 0.09 for northerly and easterly directions, respectively, indicating significant clumping. Similarly, Marshall *et al*. (1998) detected clumped distribution for a number of nematode species sampled in south eastern Scotland and likened them to the patterns seen for a diverse range of other natural phenomena.

The biological explanation for the clumped distribution of *C. impunctatus* larvae is probably the spatial structure of a variety of environmental variables, although a component of larval dispersal and also, both inter and intraspecific competition amongst the soil invertebrate fauna, in addition to female oviposition site selection, cannot be ruled out with the present data. Spatial patterns in the measured environmental variables were, however, strong and in many areas corresponded directly with those identified for *C. impunctatus* larvae. This was demonstrated in the maps of spatial density for soil percentage organic content and soil percentage water content for all three grid survey sites and also, by similarities in the semivariograms and power models for these variables and for larval counts, particularly at site 3, suggesting that variability was still increasing at the greatest distances sampled over.

Similar patterns of spatial density for larval numbers and soil percentage organic and water content were reflected in statistically significant, positive correlations between these variables, including a very strong relationship between the two soil properties. This is similar to the findings of Blackwell *et al*. (1994b), through the use of ordination analysis and multiple regression. They noted, however, that by recording a soil 'wetness index' (on an arbitrary scale from 1 to 5), that they were underestimating the importance of soil water content in these relationships. Their conclusion is confirmed by the present analyses, which showed that soil water content is a major environmental determinant of the presence of *C. impunctatus* larvae, with maximal larval numbers found in soil samples with water contents $\geqslant 90\%$. Furthermore, these data support the original hypothesis of Kettle (1961) that bogland edges of grassland were important habitats for *C. impunctatus* larvae, with fluctuating water levels perhaps having some influence on larval distribution.

In the present investigation *C. impunctatus* larval counts were also positively correlated with soil pH at all three grid survey sites. This relationship has not been identified previously for *C. impunctatus* and it reflects the intensity of the sampling scheme carried out. The spatial distribution of soil pH was not mapped since it occurred over a relatively small, lightly acidic scale. It was clear, however, that maximal larval numbers were recovered from soil samples with pH values of 5 6.5. Similarly, the distribution of *C. melleus* (Coquillet) was related to its salt marsh substrate pH (Magnon *et al*., 1990).

Mixed stands of *J. articulatus* and *J. acutiflorus* were recognized as the major plant indicator species for *C. impunctatus* aggregrations, confirming previous suggestions (Kettle, 1951; Blackwell *et al*., 1994b). The present investiga tion has also identified further important plant groups, some of which have not been highlighted previously. Positive correla tions of larval numbers with the distribution of *Sphagnum* spp. mosses at two of the survey sites agree with previous suggestions of Kettle (1961). No previous studies, however, have identified a relationship between *C. impunctatus* larvae and the deciduous shrub *M. gale*, which is found throughout the British Isles but is particularly common in bogs and wet heaths of Scotland and Ireland. Ironically perhaps, oil derived from the leaves of this plant has been shown to have repellent properties against adult biting midges in Scotland (Evans *et al*., 1996). Neither have any negative relationships been identified between plant groups and *C. impunctatus* larvae, as with the present data between all mosses apart from damp loving *Sphagnum* spp. and also *P. aquilinum.*

Through the use of geostatistical techniques to quantify the spatial variability of *C. impunctatus* larval distributions and a number of key environmental factors, it is hypothesized that larval distribution is largely habitat driven, involving a complex set of biotic factors. This results in a distinctive pattern of distribution, including a significant clumping effect. The scale over which this will occur will be related to primarily the spatial structure of the associated environmental variables. Previously, Kettle (1951) suggested that breeding sites for *C. impunctatus* were localized and that adult midge density will decrease rapidly from a breeding site, and Blackwell *et al*. (1994b) concluded that breeding areas were likely to be vast, which would reduce the possibility of midge control through targeting specific sites. The present conclu sions suggest a far more structured and predictable pattern of *C. impunctatus* larval sites. Given the present developments in geographical information systems, aiding the collection and analysis of spatially referenced data (Strickland *et al*., 1997), this makes feasible efficient targeting of control strategies aimed at larval midges. Early attempts at chemical control of *C. impunctatus* larvae were largely unsuccessful (Kettle, 1949; Kettle *et al*., 1956, 1959) but recently there have been a number of investigations of 'alternative' methods of larval control, based for example on microbial pesticides, entomo phagous nematodes and plant derived products (A. Blackwell, unpublished); future studies will incorporate the detailed information on *C. impunctatus* larval distribution given by the geostatistical techniques with these approaches to assess the practicability of midge reduction through larval control. Furthermore, the geostatistical techniques will allow studies of the underlying mechanisms determining the spatial distribution of *C. impunctatus* larvae, including their interactions with other soil organisms and the contribution of breeding site selection by the adult midges to their distribution.

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