Abundance and occupancy: use of a mesocosm to test pattern and process

by

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As with any task in life, we need to reevaluate our motives during and after completion of it. My true motive for continuing with my studies lies in the enjoyment of understanding the

question being asked and how to obtain the most 'accurate' answer possible. I would hope that this is found to be true on reading this thesis. However, my opinion remains that some questions are more important than others and require urgent answering. The Ultimate Question in life needs to be answered by each of us.

Finally, I thank my Lord Jesus Christ, my Saviour, my Rock, my Helper and Comforter, for opening a new world to me while labouring at this task.

"Who is like our God?...Nobody!"... Your yoke is easy and Your burden is light.

Title: Abundance and occupancy: use of a mesocosm to test pattern and process

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Abstract

Field-based mesocosm studies may be used as conceptual experiments to examine theoretical questions using the generated empirical data. A field-based Drosophilidae-nectarine mesocosm, comprising sun and shaded microclimate treatments arranged in a checkerboard design, was used in this thesis to examine four theoretical objectives. First, the efficacy of spatial analysis for detecting empirical pattern was examined. Natural variation weakened spatial structuring. However, hypothesis generation was shown not to be affected by this inclusion. Second, enhancing the hypothesis generating capacity of spatial methods was assessed, and confirmed to be possible, through the use of a sensitivity analysis method developed here. The method distinguished between mechanism categories (intrinsic, extrinsic and natural variation) and assessed the relative strength of each category. Next, an empirical test of the He and Gaston (2000a) parameterisation method and model to predict abundance from occupancy was conducted. Abundance estimates derived using the parameterisation method were underestimated because individuals were highly aggregated within fruit. This model and method require further exploration at fine scales for highly aggregated species. The incorporation of spatially explicit information may improve abundance predictions. Finally, the influence of spatial variation in temperature on adult body size in *Drosophila simulans* Sturtevant was investigated. The simple developmental effects of temperature differences, or the simple effects of stressful temperatures on thorax length, were overridden by interactive effects between temperature and larval density. As a result, flies attained the same final sizes in the shade and sun. Under natural conditions both mortality and non-lethal effects of temperature and/or crowding are likely to play a role in the evolution of body size. The results of this thesis provide i) an improved understanding of the

influence of natural variation on spatial pattern, ii) an additional tool for spatial hypothesis generation, iii) an empirical test of an abundance-prediction model and iv) an understanding of interactive and non-lethal effects on body size under field conditions. The thesis therefore provides empirical support for the usefulness of field-based mesocosms to examine theoretical objectives.

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CHAPTER 1

General Introduction

"Understanding patterns in terms of the processes that produce them is the essence of science…" Levin (1992)

"Community ecology is sometimes described as a discipline lacking in general rules or laws, although ecology in general is not lacking in useful generalizations (Lawton 1999)…the state of the discipline is so because of entangled abiotic and biotic factors at the scale of the community and beyond and ecology seems powerless to disentangle them…" He and Legendre (2002)

"…conceptual experiments are focused on scientific generality and the testing of general models." Englund and Cooper (2003)

The ubiquitous presence of spatial structure in biological variables

Organisms are not distributed randomly or uniformly in the natural environment. Rather, the observed distributions of organisms are structured across an environment according to the process or group of processes that are at play at the time of measurement (Legendre and Legendre 1998). This leads to the presence of spatial autocorrelation in the data (Legendre and Legendre 1998). Consequently, measurements of a variable that are taken at neighbouring sites are more similar to one another than expected by chance (positively autocorrelated) while more distant measurements of the same variable either show no autocorrelation or are negatively autocorrelated (less similar than expected by chance) (Legendre and Legendre 1998). Therefore, spatial structuring in biological variables is a fundamental component of ecosystems (Legendre and Legendre 1998). The mapping of, elucidating the causal mechanisms for, and predicting changes in, spatial variation in biological variables such as species richness, abundance, occupancy and body size have been longstanding foci of ecology. Indeed, the early works of Bergmann (1847), Raunkiaer (1934), Andrewartha and Birch (1954) and MacArthur and Wilson (1967) on distribution, abundance and body size patterns are the basis for many ecological investigations today (e.g. Hanski and Gilpin 1997, McGeoch and Gaston 2002, Frost et al. 2004, Blankenhorn and Demont 2004, Rohlfs and Hoffmeister 2004, Selmi and Boulinier 2004).

In this thesis, empirical data on the fine scale spatial structuring in a Drosophilidae-nectarine mesocosm (as measured by spatial autocorrelation) is used to investigate a number of theoretical questions (see below). How the presence of strong vs. weak autocorrelation in variables influences understanding the processes structuring variables is examined. Next, partitioning variation between structuring processes is explored and the ability of a recently developed model (He and Gaston 2000a) to predict abundance from occupancy is tested using the mesocosm data. Finally, spatial analyses are used to investigate the potential processes structuring body size in this Drosophilidae mesocosm.

Spatial structuring in biological variables occurs at all scales. At broad spatial scales, such as across continents, latitudinal and altitudinal gradients in, for example, productivity and climate may render biological variables spatially structured (Currie 1991, Kerr and Packer 1997, H-Acevedo and Currie 2003, Ashton 2004). Within local habitat patches, spatial structuring in species richness, abundance and body size may reflect behavioural (Cappuccino 1988), dispersal (Bach 1981, Duelli 1990, French et al. 2001), predation and parasitism (Winder et al. 2001, Rohlfs and Hoffmeister 2004), resource quality (Bach 1981, Brown 1984, Peng and Brewer 1994, Summerville and Crist 2001, Agnew et al. 2002), the presence and/or abundance of competitors (intra- and inter-specific) (Atkinson and Shorrocks 1981, Agnew et al. 2002), or microclimate (Levings 1983, Retana and Cerdá 2000) differences between patches. In addition, the role of these mechanisms in generating spatial structure may vary depending on the spatial and temporal framework examined, i.e. there is natural variation (*sensu* Elith et al. 2002) across individuals in the response to different mechanisms (Tobin and Pitts 2002, Papadopoulos et al. 2003).

Tools for the analysis of spatial structure

A first step towards examining ecological patterns, in an attempt to understand the generating processes, is to provide quantitative maps of biological variables (Legendre and Legendre 1998). This usually entails the use of multiple spatial analytical procedures (Dale et al. 2002, Perry et al. 2002). These procedures may then be used to generate hypotheses of the potential mechanisms affecting the observed patterns (Perry et al. 2002). Thus the identification of spatial pattern and determination of casual links to potential processes (mechanisms) are largely inseparable in spatial analyses (Liebhold and Gurevitch 2002, Perry et al. 2002, McGeoch and Price 2004). Indeed, considerable progress has been made in the field of spatial ecology (defined here as any study examining ecological patterns and processes across space) by mapping spatial patterns and

changes in patterns in an attempt to understand the processes governing their formation, i.e. the mechanistic basis of pattern generation (Liebhold and Gurevitch 2002, Perry et al. 2002, Parmesan and Yohe 2003, Blanckenhorn and Demont 2004, Wilson et al. 2004).

No lack of spatial analytical tools exist for analysis of categorical data, continuous data, data sampled in transects, regular or irregular grids and temporal replicates of spatial samples (Dale et al. 2002, Dungan et al. 2002, Keitt et al. 2002, Legendre et al. 2002, Perry et al. 2002). Further progress in the understanding of spatial patterning and the processes correlating them hinges to a large extent on the successful development of spatial analytical tools, improved computing power and programs, as well as the design of efficient sampling programs for studying and analysing spatial pattern (Legendre et al. 2002, Liebhold and Gurevitch 2002, Perry et al. 2002, Rosenberg 2004, Tobin 2004).

Issues requiring exploration

Notwithstanding the progress made in the development of spatial analytical tools, some theoretical issues remain to be explored. Although simulated data may be adopted to examine theoretical issues, simulations are regularly criticised for not mimicking biological reality (Peck 2004). By their nature, simulations are often simplistic and therefore exclude the numerous sources of natural variation present in biological systems. Empirical data is therefore required to validate the findings of simulation-based studies (Simberloff 2004). The inclusion of natural variation (*sensu* Elith 2002) in the responses of species to multiple mechanisms is likely to weaken, alter the shape, or amplify the observed spatial pattern in empirical studies (Benton et al. 2002, Einarsson et al. 2002, Didier and Porter 2003, Diniz-Filho et al. 2003, Suzuki et al. 2003). Therefore, an assessment of the implications of the inclusion of natural variation for the interpretation of patterns in empirical data is required. These implications are, on the whole, unknown (but see Ives and Klopfer 1997).

Another issue pertaining to the interpretation of the results of spatial analyses is improving the capacity of spatial analyses to generate hypotheses. The improvement and testing of enhanced hypothesis generating capacity of spatial techniques remains an active field of spatial research (Radeloff et al. 2000, Borcard and Legendre 2002, Perry et al. 2002, Borcard et al. 2004). Any advances in this field will further improve the understanding of the processes generating spatial patterns in biological variables, such as, the spatial distribution of individuals.

Although increased understanding of the spatial distribution of individuals has led to the development of models to predict the abundance of a species from its occupancy, empirical tests

of these models are limited to a few, well-studied systems (Kunin 1998, He and Gaston 2000a,b, Kunin et al. 2000). Consequently, how well these models perform at predicting abundance from occupancy across taxa and scales remains a question open to debate. However, some models (e.g. He and Gaston 2000a,b) are considered to be appropriate across a wide range of biological situations and therefore their usefulness needs to be established empirically across multiple taxa.

The use of micro- and mesocosms to generate empirical data

As mentioned above, simulation-based studies may be used to examine theoretical issues. The simplicity and high investigative control of simulation models vs. empirical data to understand natural systems is certainly advantageous (see Peck 2004). However, simulation-based studies require validation through supporting empirical studies because the former are often criticised for their lack of biological realism (Peck 2004, Simberloff 2004). The use of empirical data overcomes this disadvantage associated with simulated data, but has its own disadvantages.

Field-based empirical studies deal with data that are structured by numerous mechanisms making simple patterns unlikely (Simberloff 2004). In consequence, rendering an interpretation of field-based empirical patterns is more intricate than for simulation-based studies. Logistic constraints may also inhibit the amount of data that may be collected during a field study, especially at large spatial extents (Wiens 1989, Gaston et al. 2000, Tobin 2004). When field-based empirical data collection are insufficient, the formulation of clear hypotheses on mechanistic spatial structure may be prevented. The understanding of the system may, thus, be incomplete (Tobin 2004, but see method of MacKenzie et al. 2004 for investigating co-occurrence patterns when detection probability is low). Thus even when field patterns seem to reflect those found under simulated conditions, ascertaining the causal factor(s) underlying empirical biological variation (e.g. abundance, richness, occupancy and life history variations) remains problematic. Therefore, a compromise needs to be reached between the realism of empirical studies and the control of simulated studies.

Laboratory and laboratory microcosm studies have by and large provided the link between pure simulated and field-based investigations into biological variation (Fig. 1.1). Because of the experimental nature of microcosms, the complexity present in the system to be studied may be limited by the experimenter instead of being naturally imposed (Lawton 1995, Drake et al. 1996, Fig. 1.1). A limited number of experimental habitat types (e.g. resource quality or quantity) that are adequately replicated may be used to gauge the influence of different variables on biotic variables such as abundance, species richness or community parameters such

as productivity and persistence (Holyoak and Lawler 1996, Kassen et al. 2000, Horner-Devine et al. 2003, Jessup et al. 2004, Srivastava et al. 2004).

Notwithstanding their potential usefulness at formulating mechanistic hypotheses for community structure, competitive interactions, diversity patterns, population persistence and climate change (Connell 1961, Paine and Levin 1981, Holyoak and Lawler 1996, Davis et al. 1998, Petchey et al. 1999, Horner-Devine et al. 2003, Cadotte and Fukami 2005), microcosms have been severely criticised for their potential lack of biological reality, lack of generality, simplicity and small spatial and temporal scale (see Jessup et al. 2004, Srivastava et al. 2004 for review, Fig. 1.1). Laboratory and laboratory microcosm studies therefore suffer from many of the same criticisms levelled at simulated studies, although empirical data are collected and used (see Jessup et al. 2004, Srivastava et al. 2004, Fig. 1.1). In consequence, several recent calls for investigations into the interactions between mechanisms likely to affect biological variables under controlled field conditions have been made (Jenkins and Hoffmann 2000, Pétavy et al. 2001, Gibbs 2002, Rochette and Grand 2004).

Such controlled field conditions are obtained using mesocosms under field conditions or through the use of natural microcosms (see Srivastava et al. 2004). Here, the control of simulated and/or laboratory studies are combined with more realistic field conditions (Warren and Gaston 1997, Petersen and Hastings 2001, Relyea and Yurewicz 2002, Jessup et al. 2004, Srivastava et al. 2004, Fig. 1.1). Factors of interest can be intentionally manipulated in a controlled fashion whilst others remain a function of the “natural” mesocosm environment (Srivastava et al. 2004).

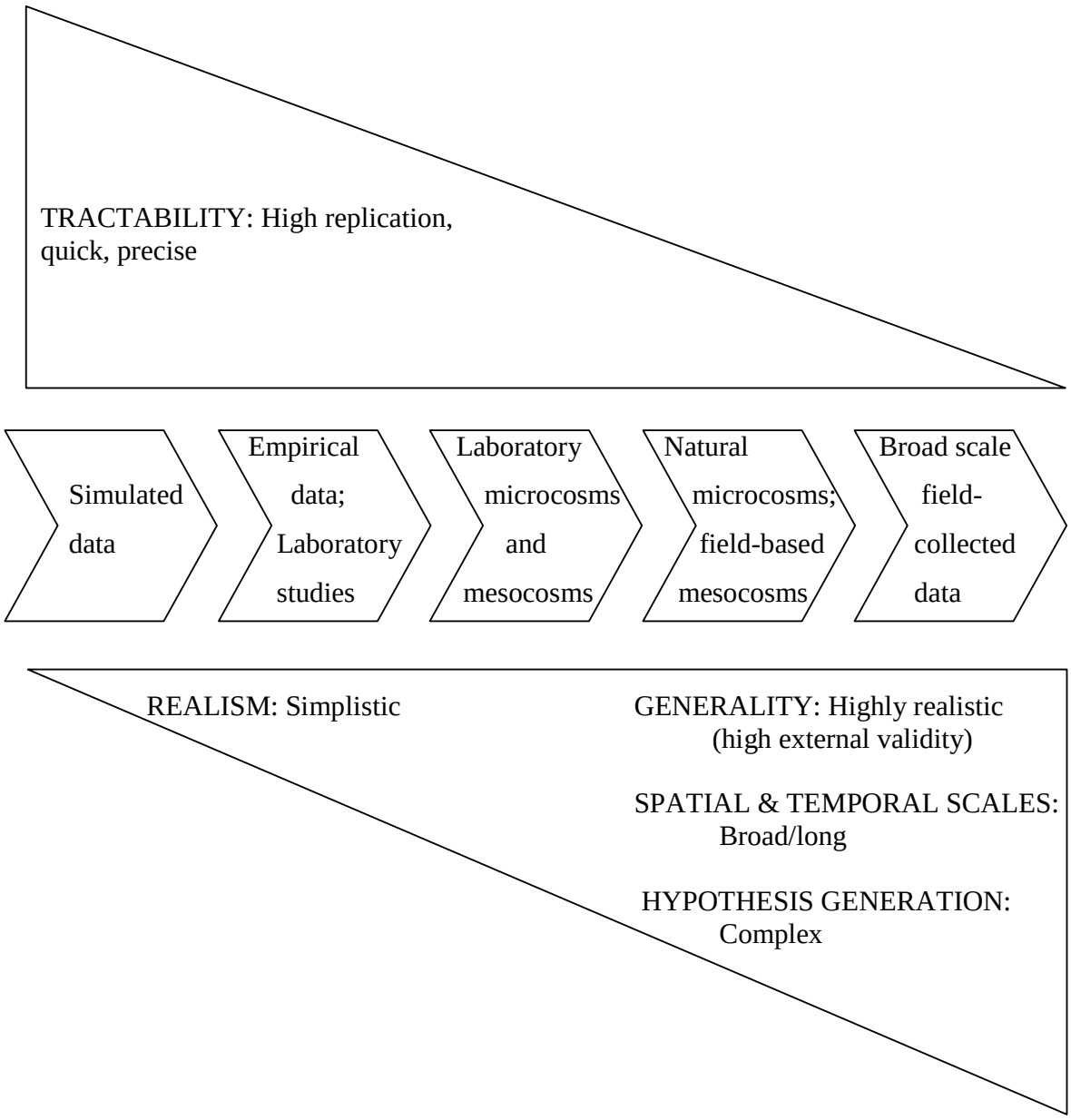

Fig. 1.1. Strengths and weaknesses of the continuum of data types that may be used to examine theoretical issues. Natural microcosms and field-based mesocosms form an essential link between theoretical laboratory studies and the validation of theoretical models in complex natural systems (see Englund and Cooper 2003, Jessup et al. 2004, Srivastava et al. 2004).

Much of the criticism leveled at "home-grown" assemblages is negated when using field-based mesocosm vs. laboratory microcosm studies (but see Davis et al. (1998) for a realistic laboratory-based mesocosm using three species of *Drosophila* and a parasitoid). For example, the spatial dimensions of mesocosms are larger than microcosms. More complex eukaryotic organisms are allowed to colonise the habitat naturally instead of, for example, microorganisms that are selected *a priori* to interact. In addition, mesocosms generate assemblages that are biologically realistic in terms of species number and composition (i.e. they are unaffected by species identity effects; see Benedetti-Cecchi (2004) on experimental design modifications to separate these and other effects). The organisms inhabiting field-based mesocosms may be manipulated to reduce the complexity present in natural systems. Field-based mesocosms therefore display many of the advantages of natural microcosms that have been suggested to be (more) useful than laboratory-based microcosms at understanding the processes generating ecological patterns (see Srivastava et al. 2004, Fig. 1.1). Finally, abundance and species richness can be accurately measured and no factors beyond the extent of the mesocosm study arena are likely to be structuring the observed patterns (bar historical effects in the natural landscape in which the mesocosm is situated, and shared evolutionary history, Srivastava et al. 2004).

Although the spatial dimensions of mesocosms are larger than those of microcosms, the spatial and temporal scales of most micro- and mesocosm studies are short and small respectively in terms of natural systems. However, this is usually not a problem in the context of theoretical examinations, i.e. conceptual experiments (Englund and Cooper 2003, Jessup et al. 2004, Srivastava et al. 2004). Here, the processes of interest need to be reproduced rather than creating an exact replica of a particular system (Englund and Cooper 2003). Model systems, such as microbes in jars (see Jessup et al. 2004), the Ecotron facility (Lawton 1996) and natural microcosms (e.g. aquatic communities in pitcher plants, Srivastava et al. 2004), can be used to test general models in which the process of interest is captured. Therefore, as for natural microcosms, field-based mesocosms may be considered "model systems", i.e. conceptual experiments, for testing theoretical questions using empirical data (Srivastava et al. 2004).

On the other hand, system-specific experiments are meant to mimic a particular natural system (Englund and Cooper 2003). Processes and environmental conditions need to be realistic to extrapolate the results to field situations (Englund and Cooper 2003). Some field-based mesocosms are inherently able to double-up as both conceptual and system-specific experiments. For example, field-based Dipteran communities inhabiting necrotic resources such as mushrooms, fruit and carrion (Beaver 1977, Atkinson and Shorrocks 1984, Worthen et al.

1994,Worthen and Haney 1999) are able to test theoretical questions while limited or no scaling up is required to understand the community level processes that may be at work.

A Drosophilidae mesocosm as a model system

The family Drosophilidae is a diverse taxon comprising approximately 3000 species worldwide (Remsen and O'Grady 2002, de Medeiros and Klaczko 2004). Hawaii is particularly diverse with an amazing sixth of all described drosophilid species found on the archipelago (Remsen and O'Grady 2002). The taxonomic diversity of drosophilids stems, at least in part, from their ability to occupy a wide variety of ecological habitats and niches. Most drosophilids breed and feed on decaying plant matter (mainly fruits) and some utilise mushrooms and slime fluxes exclusively (Shorrocks 1982, Ashburner 1989).

It is therefore not surprising that drosophilids have a long history of use as model study organisms in evolutionary, genetic and physiological studies (Srivastava et al. 2004). Much is known, mainly from laboratory studies, about the phylogenies, life histories and physiological constraints likely to limit the species in the family (Remsen and O'Grady 2002, Hoffmann et al. 2003, David et al. 2004, Gibert et al. 2004). Indeed, drosophilids may yet be the most well studied group of organisms in the world. For example, one of the first genomes ever to be mapped was of a drosophilid, *Drosophila melanogaster* Meigen (Remsen and O'Grady 2002, Celniker and Rubin 2003).

Despite this plethora of information on the species in this family, far fewer studies of any of these species have been undertaken in the field to investigate ecological patterns and processes (e.g. Atkinson and Shorrocks 1984, Atkinson 1985, Nunney 1990, Worthen et al. 1994, Worthen and Haney 1999, Mitsui and Kimura 2000). This is particularly surprising given a) their diversity, b) that many of these species (e.g. *D. melanogaster* and *D. simulans* Sturtevant) are cosmopolitan in their distributions making study site establishment effortless and c) that the species are located in habitats with defined boundaries (e.g. fruit, mushrooms) facilitating easy sampling and adequate replication (Atkinson and Shorrocks 1984, Finn 2001). On the whole, the use of drosophilid systems as models for both conceptual and system-specific ecological experiments (Englund and Cooper 2003) remains untapped.

The advantages of systems used by Drosophilidae to investigate certain conceptual (theoretical) issues are also evident. First, drosophilid assemblages are known to be spatially structured (e.g. Shorrocks and Rosewell 1987). These assemblages therefore provide empirical data for investigations into the ability of spatial analytical procedures to detect and quantify

spatial pattern and for testing alternative methods to improve hypothesis-generating capacity. Rosewell et al. (1990) found that the negative binomial distribution (NBD) adequately described the distribution of *Drosophila* assemblages, and the NBD is not unusual for invertebrate assemblages (Sevenster 1996). Using empirical data collected from such an assemblage is therefore an excellent test of the ability of the He-Gaston model (He and Gaston 2000a) to predict the abundance of invertebrate assemblages from occupancy measures as it uses the NBD during parameter estimation. Moreover, Drosophilidae have an extensive history of use in laboratory and correlative field investigations into life history variables (e.g. Sevenster and Van Alphen 1993, Partridge et al. 1994, Nunney 1996, Jenkins and Hoffmann 2000, Pétavy et al. 2001, Gibbs 2002). Recently, a call has been issued for controlled field-based studies of the variation in life history and physiological parameters under field conditions because field and laboratory results may differ (Jenkins and Hoffmann 2000, Pétavy et al. 2001, Gibbs 2002). Comparison of field-based results with laboratory studies, where all (or most) factors are controlled, are required to affirm the validity of the conclusions drawn from laboratory studies with respect to natural systems. The use of a controlled field-based Drosophilidae-nectarine mesocosm is therefore able to shed light on questions pertaining to life history variation in a spatially structured environment.

The field-based drosophilid mesocosm

This study uses all of the above advantages associated with drosophilid systems and field-based mesocosms to provide empirical data from a model system to investigate theoretical questions. In particular, the study had the following objectives:

- First, the ability of spatial analytical procedures to detect spatial pattern, given the high levels of natural variability present in biological systems that is likely to weaken, amplify or obscure spatial pattern (autocorrelation), was examined (Chapter 2). The rare and common species in the assemblage were expected to respond similarly to the imposed treatment. However, the response of the rare species, per capita, was expected to be weaker than the common species (see Chapter 2 for rational). A comparison is therefore made between rare and common species to assess the ability of spatial analyses to distinguish spatial randomness (zero autocorrelation) from biologically meaningful, but weak, spatial pattern (autocorrelation) (Chapter 2). An appraisal of published Moran's *I* autocorrelation values are used to confirm the validity of the assumption that biological spatial pattern (autocorrelation) should, in general, be weak (Chapter 2).

- The next step in the development of spatial tools is the enhancement of hypothesis-generating capacity of spatial analytical procedures. In Chapter 3, a variation partitioning technique is developed to maximise the hypothesis-generating capacity of spatial procedures. Both graphical and statistical approaches are used in an attempt to enhance the understanding of the mechanistic basis of the observed spatial patterns (Chapter 3). That is, by partitioning the spatial structure into different categories of structuring mechanisms, a better understanding of the relative importance of these mechanism categories towards generating observed autocorrelation may be gained.
- In Chapter 4 the accuracy of the He-Gaston model (He and Gaston 2000a) to predict abundance from occupancy for this drosophilid mesocosm is examined. The potential explanations behind the failure of the He-Gaston model to accurately predict the abundances of the species inhabiting this mesocosm are elucidated.
- Chapter 5 investigates spatial pattern in a measure of body size (thorax length). Simple and interactive mechanistic hypotheses for explaining body size variation are proposed and tested by examining spatial pattern in thorax length.

Each chapter is presented as a research paper and consequently some of the methods and references overlap.

A field-based experimental mesocosm was established at the University of Pretoria's Experimental Farm in Pretoria, South Africa (25º45.178"S, 28º15.293"E; Fig. 1.2) in November 1998. The mesocosm was divided into six equal plots. Three of the plots in alternate rows of the two columns were artificially shaded with 80 % shade netting (Fig. 1.3). The imposed treatment introduced heterogeneity in microclimate to the mesocosm. This reflected a level of complexity likely to be found in natural systems and provided a basis for capturing the natural processes in the system, thereby establishing the mesocosm's utility as both a conceptual and system-specific experiment.

Drosophilids naturally occurring in the urbanised area were allowed to colonise an abundant necrotic fruit resource (*Prunus persicae* Miller variety nectarina). Twenty-three species have been identified utilising resources in urban areas in Pretoria and Johannesburg, South Africa (McEvey et al. 1988). Because many drosophilids are resource specialists and a single food type was used, species richness in the mesocosm was expected to be low. In this study at least six species were identified but specimens belonging to the genus *Zaprionus* could not be identified to species level. The identified species were: *Drosophila simulans* Sturtevant, *D. melanogaster* Meigen, *D. busckii* Coquillett, *D. buzzatii* Patterson and Wheeler, *Zaprionus*

morphospecies group 1 (*Zaprionus* msg 1) (may include both *Z. tuberculatus* Malloch and *Z. sepsoides* Duda) and *Zaprionus* morphospecies group 2 (*Zaprionus* msg 2) (may include both *Z. vittiger* Coquillett and *Z. indianus* Gupta) (McEvey et al. 1988). The number of identified species meets the expectation for the number of drosophilids comprising a guild (Shorrocks and Rosewell 1987).

D. simulans was numerically dominant on all of the sampling occasions in November 1998 (Fig. 1.4). The abundances of the remaining species varied substantially between species (Fig. 1.4). Many fruit yielded no flies. Therefore, the Drosophilidae assemblage structure was very similar to the structure that has been found for other dipteran assemblages associated with ephemeral resources (e.g. Atkinson and Shorrocks 1984, Sevenster and Van Alphen 1993). The high variability in relative abundance between species and in occupancy of resources across species appears typical of such assemblages (Beaver 1977, Atkinson 1985, Shorrocks and Rosewell 1987).

Therefore, the drosophilid-nectarine mesocosm used here reflected natural drosophilid assemblages in terms of species richness, abundance and occupancy. In addition, the imposed microclimatic treatment introduced heterogeneity into the field-based mesocosm system while reducing the complexity that is present in field-based studies. Drosophilidae systems may therefore be viewed as “natural microcosms” (see Srivastava et al. 2004) that are able to capture essential components of both conceptual and system-specific experiments (Englund and Cooper 2003).

Fig. 1.2. Covered experimental mesocosm that was established on the University of Pretoria's Experimental Farm (see text for details).

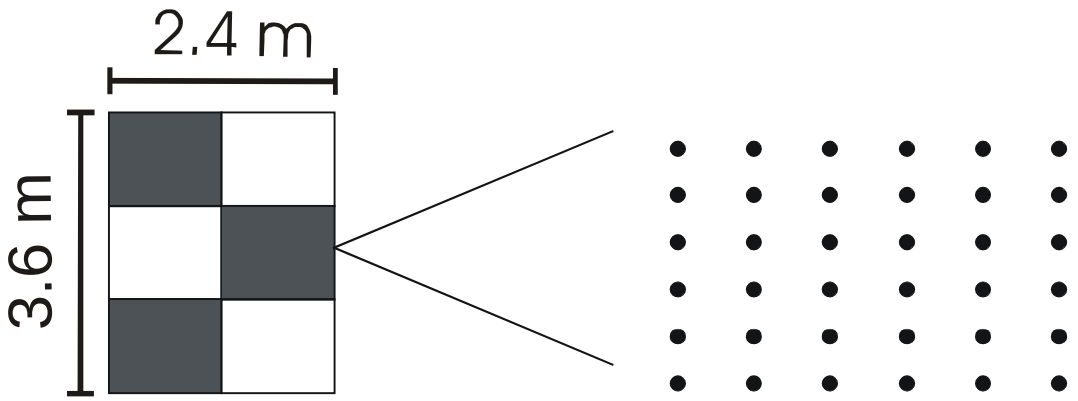

Fig. 1.3. Experimental plot depicting the layout of decaying fruit. Dark blocks represent plots that were shaded with 80 % shade netting. Each plot contained 36 nectarines spaced 20 cm apart in a regular grid as on the right hand side of the figure.

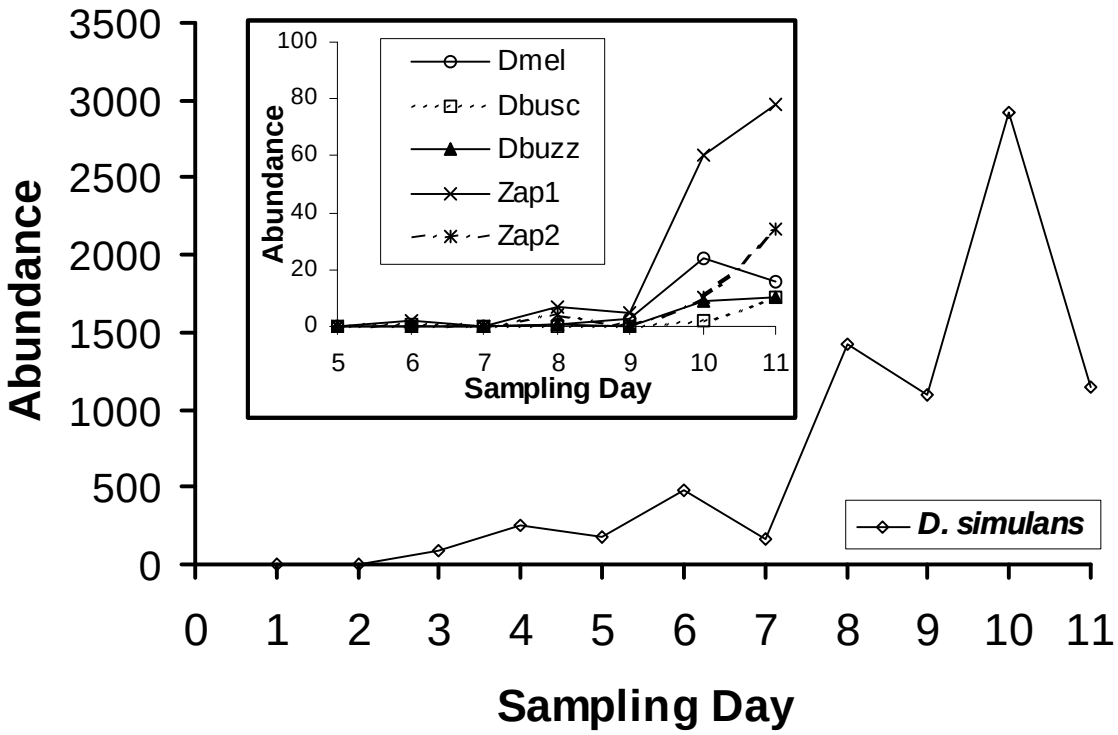

Fig. 1.4. Abundances of the six recorded species (or morphospecies groups) during November 1998 (Dsim= *Drosophila simulans*, Dmel = *D. melanogaster*, Dbusck = *D. busckii*, Dbuzz = *D. buzzatii*, Zap1 = *Zaprionus* morphospecies group 1, Zap2 = *Zaprionus* morphospecies group 2).

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CHAPTER 2

The detection of spatial structure in populations and assemblages: an empirical case study

Introduction

A central theme in ecology is the variation in species richness and abundance across space. One tool for generating hypotheses for the mechanisms causing spatial pattern is the application of spatial analysis to empirical data (Liebhold and Gurevitch 2002, Perry et al. 2002). Recently, the relative merits of a variety of analytical approaches to quantifying this spatial pattern have been discussed (Koenig and Knops 1998, Legendre and Legendre 1998, Dale et al. 2002, Perry et al. 2002, Plotkin et al. 2002). However, determining an appropriate and effective analytical approach remains a challenge, and the options are numerous (Liebhold and Gurevitch 2002). Although the factors likely to influence both the choice of method and the output obtained have been outlined (Dale et al. 2002, Dungan et al. 2002, Keitt et al. 2002, Perry et al. 2002, Xu and Madden 2003), understanding the interpretation of the outputs of alternative methods remains incomplete. For example, many spatial studies are based on simulated datasets (Sokal and Wartenberg 1983, Legendre and Fortin 1989, Radeloff et al. 2000, Laakso et al. 2001, Anderson and Neuhauser 2002, Legendre et al. 2002, Fortin et al. 2003) and, therefore, the implications of the inclusion of natural variation for the interpretation of patterns in empirical data are largely unknown. As a result two issues require further exploration: First, to what extent is it possible to detect (and therefore quantify) spatial pattern in empirical data given the often high levels of natural variation? Second, how useful are the available techniques for generating realistic hypotheses? The first issue (the influence of natural variation) is examined here.

Simulation-based approaches have regularly been used to examine the relationship between the results of spatial analytical procedures (geostatistical and spatial methods, Liebhold et al. 1993, Liebhold and Gurevitch 2002) and spatial pattern (the spatial realisation of a process or processes, Fortin et al. 2003) (Sokal and Wartenberg 1983, Legendre and Fortin 1989, Radeloff et al. 2000, Anderson and Neuhauser 2002, Legendre et al. 2002). In general, such studies generate artificial data surfaces to which spatial analysis is then applied (e.g. Radeloff et al. 2000, Legendre et al. 2002). These simulation studies have shown that geostatistical methods effectively detect spatial pattern in artificial surfaces, albeit to some extent dependent on the regime used to sample the surface (see Legendre et al. 2002). For example, Radeloff et al. (2000) showed that periodicity in simulated environmental surfaces was detected as such by

spatial autocorrelation analysis. Legendre and Fortin (1989) and Legendre and Legendre (1998) also demonstrate that spatial autocorrelation effectively detects spatial structure in simulated environmental surfaces, such as gradients, bumps, waves and steps. Therefore, simulation-based studies have demonstrated that geostatistical methods effectively describe spatial pattern in artificial surfaces.

However, simulated data are generally deterministically structured and the simulated surface is known *a priori* (see Radeloff et al. 2000, Legendre et al. 2002). On the other hand, the pattern in empirical data is often generated by multiple mechanisms operating across a range of spatial scales (Thrush 1991, Legendre and Legendre 1998, Legendre et al. 2002, Ni et al. 2003). These multiple mechanisms lead to complex biological responses (e.g. Einarsson et al. 2002) that differ in both space and time (Austin 2002, Elith et al. 2002, Brewer and Gaston 2003, Ni et al. 2003, Xu and Li 2003, Laakso et al. 2004, McGeoch and Price 2004, Tobin 2004). Natural variation (*sensu* Elith et al. 2002) is therefore likely to alter the quantified spatial response (i.e. geostatistical output) (Elith et al. 2002). Consequently, the spatial responses observed in empirical data may be weak as a result of either reduced strength or altered shape of the response, responses from different mechanisms acting in opposing directions, or amplification of the response by multiple mechanisms (Ranta et al. 2000, Benton et al. 2001, 2002, Laakso et al. 2001, Einarsson et al. 2002, Didier and Porter 2003, Diniz-Filho et al. 2003, Suzuki et al. 2003). Therefore, simulation-based surfaces are likely to be overly simplistic and may often be structured more strongly than empirical data (Benton et al. 2001, 2002, Austin 2002, Elith et al. 2002, Shen et al. 2004), whereas in empirical studies pattern is likely to be weaker and more difficult to detect, and hypothesis generation based on the output of spatial analyses therefore more complex.

A complication in the identification of spatial pattern arises when the spatial pattern is weak (Koenig 1999). Spatial analytical procedures may have insufficient power to identify significant pattern when the quantified response is weak (Legendre and Legendre 1998). For example, autocorrelation analysis would reveal that the pattern is not significantly different from random (Dessaint et al. 1991, Legendre and Legendre 1998). Therefore, no spatial pattern is said to be present although this conclusion may result from a weak response and not necessarily because there is no spatial structure present in the data. In addition, Koenig (1999) argued that weak, significant structures are actually biologically meaningless and, for example, an artefact of large sample sizes. Distinguishing between weak, yet biologically meaningful pattern and the absence of pattern may therefore be difficult.

One possible approach to exploring this problem further is to compare the responses of rare and common species in a system where both sets of species are known to be spatially structured by the same mechanisms. The spatial output of rare species (those that do occur sufficiently frequently to warrant analysis, see Legendre and Fortin 1989) is likely to be weaker than common species (see Judas et al. 2002, Overton and Levin 2003), because species with low abundances (rare species) are likely to occupy fewer localities than species with higher abundances (common species, *sensu* Gaston 1994). The examination of spatial pattern in rare species may thus clarify the degree to which spatial pattern identification and interpretation is affected by weak spatial responses. To date few comparisons of outputs of different strengths have examined the presence, detectability and biological significance of quantified spatial structure (for examples see Dessaint et al. 1991, Overton and Levin 2003).

To further examine the merits and interpretation of the outcome of spatial analyses, a compromise needs to be reached between overly simplistic, simulated representations of the environment and those that are realistic in their complexity. That is, an approach that includes some but not all of the complexity that is likely to obscure, weaken or amplify spatial patterns in natural systems. One such compromise is the use of experimentally generated data, where it is possible to control some of the environmental variability, and explicitly measure that component known to be important to the system in question. For example, mesocosms generate assemblages similar to those expected under field conditions by incorporating realistic variation without the additional complexity involved in mensurate, field-based studies (Warren and Gaston 1997, Relyea and Yurewicz 2002, Warren et al. 2003). To date, use has not been made of experimentally generated empirical data, or mesocosms, to examine the relationship between environmental and biological response surfaces and geostatistical output after imposing a known spatial pattern, despite the benefits of doing so. This is done here.

The mesocosm generated a heterogeneous environmental surface and this environment was colonised naturally by an assemblage of vinegar flies (Diptera: Drosophilidae). The objectives were 1. to determine if the observed spatial autocorrelation pattern accurately reflected predicted pattern, and 2. if weak autocorrelation patterns are useful for generating hypotheses. To examine the strength of spatial pattern in empirical studies, and to confirm that autocorrelation coefficients (*I* values) obtained for the biological variables in the mesocosm are realistic (i.e. fall within the range of published *I* values), published autocorrelation coefficients (*I* values) were summarised and compared with those achieved for biological variables in the mesocosm used in this study.

Materials and Methods

Study organisms and sampling design

An urban Drosophilidae assemblage that inhabits decaying fruit (in this case nectarines (*Prunus persicae* Miller: Rosaceae)) was used in a mesocosm consisting of 216 similarly-sized fruit (Warren et al. 2003). The fruit were washed and weighed at the start of the experiment. The variation in fruit mass was small and not significantly different between sun (Mean ± S.E. = 58.59 g ± 1.09) and shade (Mean ± S.E. = 59.08 g ± 1.07) plots ($t = 0.35$, $df = 214$, $P = 0.72$). Therefore, resource availability (fruit mass) was assumed to play no role in explaining assemblage differences between treatments. Five additional nectarines were randomly selected for insecticide residue tests and were found to have no detectable levels of residues of the following chemicals used in the local soft fruit industry: organophosphates (Dimethonate, Malathion, Triazophos), organochlorides (γ-BHC, β-Endosulphan, Endosulphan sulphate) and pyrethroids (Cypermethrin, Deltamethrin) (South African Bureau of Standards).

A wire table (2.4 m x 3.6 m; 0.7m high) was placed out at the University of Pretoria's Experimental Farm in Pretoria, South Africa (25°45.178"S, 28°15.293"E). The table was located inside a cage covered with pigeon wire to exclude birds, fruit-piercing moths and large wasps, while allowing the flies ready access to the fruit. Ants were also excluded by the application of grease to the table legs. The table (hereafter the 'study arena') was divided into six equal plots with each plot supporting 36 nectarines (n = 216 nectarines)[1] (Fig. 2.1). The mesocosm, albeit at a smaller scale, was thus typical of the layout and symmetry found in agricultural ecosystems and characterised by few patches of a single type (Perry et al. 2002). Three of the plots were artificially shaded with 80 % shade netting to impose variation in microclimate across the study arena, and thereby add a level of spatial heterogeneity in microclimate to the experiment (Fig. 2.1). Six copper-constantan thermocouples were placed 1 cm under the top skin of six nectarines (three fruit were exposed to the sun and three were shaded) to measure the temperature of the fruit every 10 minutes for the duration of the experiment (recorded by a Campbell Scientific CR10 datalogger using PC208 software for programming and data capture). The experiment ran from 6 November to 1 December 1998 by which time the fruit were shrivelled and dry and black in colour.

[1] There were two reasons for deciding on this experimental design (n=216): a) Legendre and Fortin (1989) state that a minimum of 30 sampling points are required to draw a correlogram. We preferred >30 points per sun/shade plot, b) It was physically impossible to reach the fruit in the centre of the study arena to replace the sand and to water it if plot size was > 6X6 fruit wide, i.e. 6 plots of 36 fruit = 216 fruit.

The nectarines were placed 20 cm apart in a regular grid of 12 x 18 fruit (Fig. 2.1). Each fruit was placed on coarse plastic mesh in the centre of a round plastic container (~ 15 cm diameter and 8 cm deep) containing washed, moist sand. A fixed volume of water was sprayed into the containers every day to ensure that the sand remained moist. *Drosophila* larvae drop down into the substrate under the fruit and pupate at 4.5 to 8.5 days for flies at 25 °C and 80 % RH (Sevenster and Van Alphen 1993). Larvae were therefore allowed to pupate in the sand beneath the fruit before being collected in plastic jars and being taken to the laboratory. Starting from the fifth day after exposure of the fruit, the sand containing the fly pupae under the nectarines was removed and placed in 350 ml plastic jars every second day for 25 days (eleven sampling occasions). Fresh, moist sand was immediately placed in the containers under the fruit at each collection. The plastic jars were taken to the laboratory and the emerging flies identified according to McEvey et al. (1988). At least six Drosophilidae species were found. However, specimens belonging to the genus *Zaprionus* could not be identified to species level. The identified species were: *Drosophila simulans* Sturtevant, *D. melanogaster* Meigen, *D. busckii* Coquillett, *D. buzzatii* Patterson and Wheeler, *Zaprionus* morphospecies group 1 (*Zaprionus* msg 1) (may include both *Z. tuberculatus* Malloch and *Z. sepsoides* Duda) and *Zaprionus* morphospecies group 2 (*Zaprionus* msg 2) (may include both *Z. vittiger* Coquillett and *Z. indianus* Gupta) (McEvey et al. 1988).

Because of their small size, the body temperatures of the drosophilids are expected to approximate the temperature of the environment in which they are found (Stevenson 1985). Environments with high ambient temperature such as those recorded for exposed fruit are unlikely to be suitable habitats for the development and survival of these flies (Feder et al. 1997a, Feder and Krebs 1998). The recorded maximum temperatures of the fruit exposed to the sun (above 50 °C), and the duration of exposure to temperatures above 37 °C (range across thermocouples Sun: 31-68 hr; Shade: 0-12 hr), are sufficient to kill *Drosophila* larvae (Feder and Krebs 1998, Worthen et al. 1994), i.e. the treatment is likely to lead to higher mortality in sun than shade plots. Although females are unable to determine past thermal history of a fruit, females avoid fruit if these are hot at the time of oviposition (Feder et al. 1997b). Therefore, females are likely to avoid hot sun fruit at certain times of the day when fruit temperatures are above 37 °C, further decreasing abundance and species richness in sun plots. Different fly species are expected to respond to these abiotic conditions in a similar manner (Feder and Krebs 1998). Therefore, abundance and species richness are expected to be lower in high temperature (sun) environments (Feder et al. 1997a,b, Feder and Krebs 1998, Worthen and Haney 1999). The

biological response surfaces revealed that species were occurring at higher abundances in the shaded than sun plots (Fig. 2.2). Therefore, the desired pattern of higher abundance, occupancy and species richness in shaded plots was achieved by the experimental design (Table 2.1). Although species composition was similar between treatments, total abundance was almost six times higher in shade than in sun plots (Table 2.1).

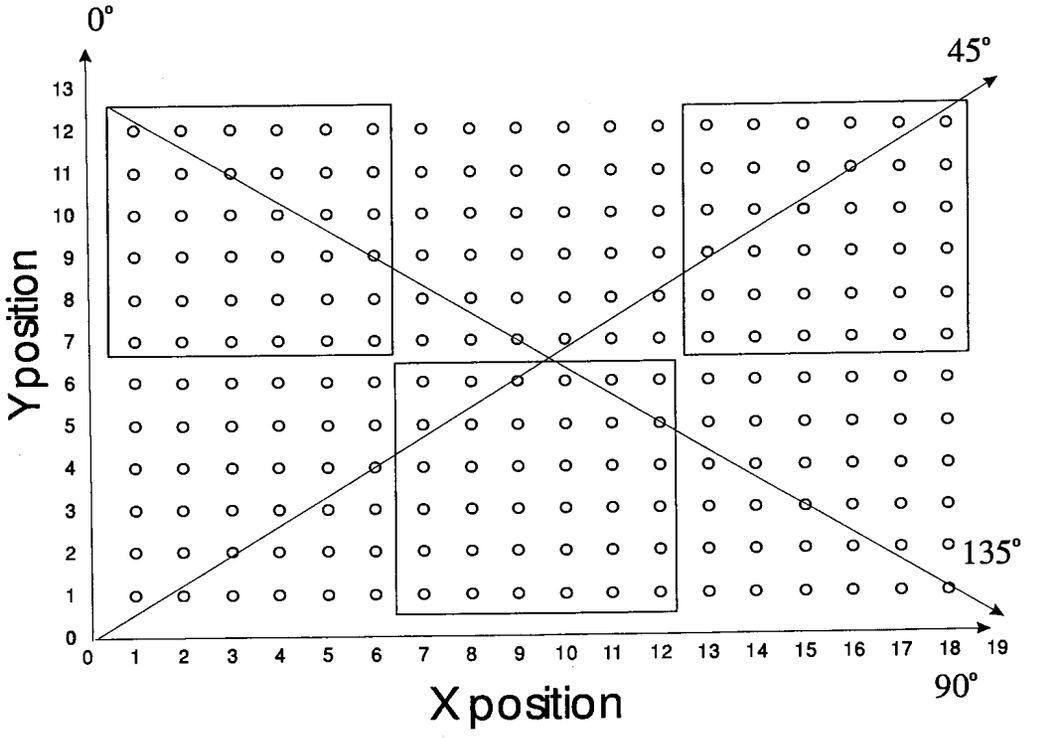


Fig. 2.1. Experimental plot (study arena) depicting the layout of decaying fruit (X position = 3.6 m x Y position = 2.4 m). Thin, solid rectangular outline around groups of fruit represent plots that were shaded with 80 % shade netting. Unblocked circles represent fruit exposed to the sun. Each plot contained 36 nectarines spaced 20 cm apart in a regular grid. Arrows depict directions used for directional correlogram analysis (0^0, 45^0, 90^0 and 135^0).

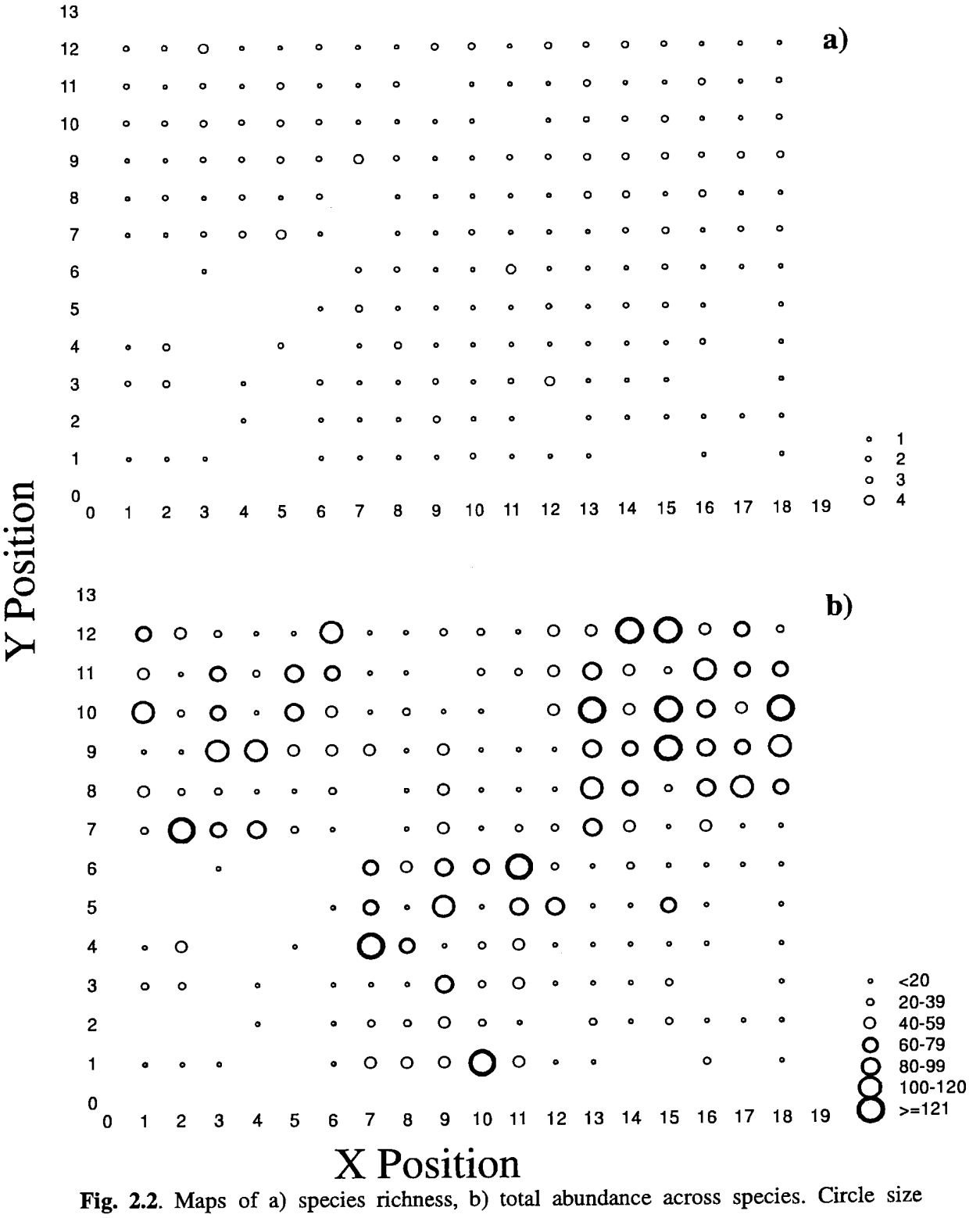


Fig. 2.2. Maps of a) species richness, b) total abundance across species. Circle size represents species richness or abundance of flies in individual fruit (Continued on following page).

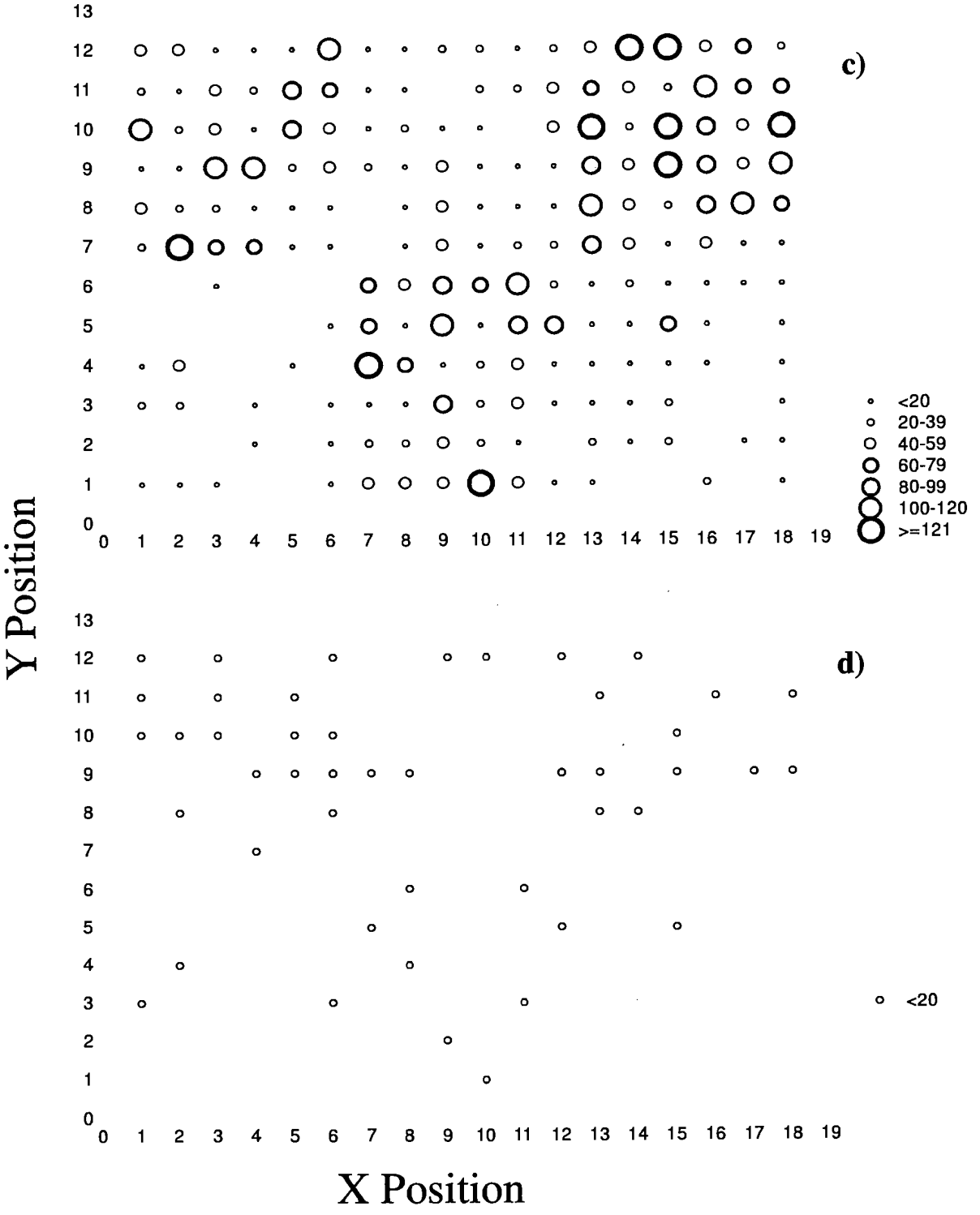


Fig. 2.2 continued. Maps of c) *D. simulans* abundance and d) *Zaprionus* morphospecies group 1 abundance. Circle size represents abundance of flies in individual fruit.

Table 2.1. Number of fruit occupied, total and mean (± S.E.) abundance of species in the assemblage, differences in total abundance (Mann-Whitney U = 1248, n = 108, P < 0.001) and species richness (Mann-Whitney U = 2901.50, n = 108, P < 0.001) between sun and shade plots and significant cross-correlation coefficients (± S.E.) for the environmental surface with total abundance, species richness, occupancy, *D. simulans* and *Zaprionus* msg 1 for distance class 1 (zero to 2 adjacent fruit) (all significant at P < 0.05).

Species	Sun plots (n = 108 fruit)			Shade plots (n = 108 fruit)			
	No. fruit occupied	Abundance Total	Mean ± S.E.	No. fruit occupied	Abundance Total	Mean ± S.E.	Cross-correlation coefficient (± S.E.)
D. simulans	75	1133	10.49 ± 1.36	107	6485	60.05 ± 5.03	0.93 ± 0.28
Zaprionus msg 1	10	21	0.19 ± 0.09	36	130	1.20 ± 0.22	0.88 ± 0.28
D. melanogaster	5	6	0.06 ± 0.03	21	38	0.35 ± 0.09	-
Zaprionus msg 2	10	22	0.20 ± 0.08	19	27	0.25 ± 0.06	-
D. buzzatii	0	0	-	4	20	0.19 ± 0.12	-
D. busckii	1	1	0.01 ± 0.01	6	11	0.10 ± 0.05	-
Total abundance		1183	10.97 ± 1.41		6711	62.31 ± 5.09	0.93 ± 0.28
Total species richness		5	0.94 ± 0.08		6	1.79 ± 0.09	0.78 ± 0.28
Occupancy	76			107			0.86 ± 0.28

Quantifying spatial pattern using correlograms

The spatial structure of the environmental surface was quantified using the rectangular structure of the experiment (the spatial position of all 216 fruit). Each fruit was coded (0,1) as occurring in either a sun (1) or shade (0) plot in the experiment (Fig. 2.1), and these data were used to depict the spatial structure of the environmental surface. The abundance and species richness data for each fruit was pooled across the eleven sampling occasions, to examine the quantified spatial pattern for the duration over which the fruit resource was available. Pooling spatial samples that have been taken over many sampling occasions may contribute to the detection of significant spatial pattern (this is a form of natural variation termed 'spatiotemporal' by Ives and Klopfer (1997)). However, calculating species abundances over numerous sampling occasions, and deriving occupancy from these abundances, is standard procedure at larger sampling scales (see, for example, Brown et al. 1995, Harrison et al. 1997). Therefore, a total abundance value (all species pooled) and abundance values for each species separately over the entire sampling period were obtained for each fruit. These values were then used to quantify and compare patterns in the biological response surfaces of the assemblage variables (species richness, total abundance) and individual species abundances across the study arena (i.e. the biological variables). The effect of a spatiotemporal mechanism (pooling spatial samples from multiple sampling occasions) on the strength of the autocorrelation output was examined by drawing omnidirectional correlograms of species richness, total abundance and *D. simulans* abundance for sampling occasion 10 alone, when abundance was highest (Warren et al. 2003). *Zaprionus* msg 1 occupancy was below the minimum of 30 occupied fruit for this sampling occasion and the correlogram for this species was therefore not drawn. Because all emerging flies were counted, the quantified structures are those resulting from the true abundances of the species constituting the assemblage with no sampling effect.

Two levels of spatial structuring in the fly assemblage were expected. First, a response to the microclimatic heterogeneity induced by the mesocosm (sun and shade plots) and avoidance of sun fruit by ovipositing females when fruit are hot, and second, spatial structuring within individual (aggregation) and across adjacent fruit (a consequence of fly oviposition behaviour) (Feder et al. 1997 a,b, Heard and Remer 1997, Feder and Krebs 1998, Remer and Heard 1998, Worthen and Haney 1999, Warren et al. 2003). Females of different drosophilid species are likely to respond similarly to the abiotic conditions (Krebs 1999). Therefore, structuring of rare and common species by the same mechanisms is expected to yield similar spatial outputs that differ only in the strength of the response.

Directional correlograms (standardised covariograms) and omnidirectional correlograms (Moran's *I*) were used to illustrate the results of the spatial analyses across the study arena (using S+SPATIALSTATS module, Kaluzny et al. (1998) and SAAP-PC Version 4.3, Exeter Software, Wartenberg (1989) respectively). The construction of directional correlograms for this dataset are likely to be more accurate at detecting spatial pattern (see below), but they require a much larger sample size than omnidirectional correlograms to test for significance (e.g. 4950 point pairs in an omnidirectional correlogram reduces to 450 point pairs for a directional correlogram in a 10 x 10 grid, Dungan et al. 2002). Therefore, directional correlograms (standardised covariograms) were used to confirm the predicted pattern, for the environmental surface and total abundance, and omnidirectional correlograms were used to examine the spatial structure and to test for significance (Bonferroni-corrected) in autocorrelation coefficients across the study arena (Legendre and Legendre 1998). Although Radeloff et al. (2000) recommend using directional correlograms when dealing with grid and checkerboard patterns, no anisotropy (directional pattern) in abundances and species richness across the study arena was apparent from initial raw data plots of the biological response surfaces from this study (Fig. 2.2). However, omnidirectional correlograms combine the spatial pattern from multiple directions into one correlogram and some resolution of the spatial pattern in a checkerboard design will be lost (see Radeloff et al. 2000).

Directional correlograms were constructed in four directions (0^0, 45^0, 90^0 and 135^0; Fig. 2.1) with each class representing 0.27 m. In square sampling areas, the x-intercept (where the correlogram becomes negative or zero) approximates the length of one side of the square (Sokal and Wartenberg 1983) and should therefore approximate six fruit neighbours for this experimental design. The biological variables were thus expected to be positively autocorrelated for distance classes 1--4 and 9--13 and negatively autocorrelated for classes >4--<9, corresponding to the sun and shade plot sizes in the experiment for the 0^0 and 90^0 directions (although for 0^0 autocorrelation would not extend beyond class nine, encompassing the size of two plots in that direction, Fig. 2.1). The first four distance classes correspond to the size (width) of a microclimate treatment plot (six fruit neighbours). Distance classes >4--<9 correspond to the distance between adjacent plots (different treatments) (7--12 fruit neighbours), while the distance classes 9--13 correspond to the distances between plots of the same treatment (13--18 fruit neighbours). The 45^0 and 135^0 directional correlograms will not correspond as closely to this prediction, because plot structure is different (fewer fruit between plot types) in these directions (see Fig. 2.1).

Omnidirectional correlograms were constructed using 15 distance classes (obtained using Sturge's rule[2], Legendre and Legendre 1998) and equal distance intervals (each class therefore represented 0.27 m; equivalent to approximately two adjacent fruit), and the number of point pairs in each distance class thus varied. It is recommended that only half the total distance of the correlogram should be interpreted (Rossi et al. 1992, Kaluzny et al. 1998). However, the correlograms fulfilled the minimum requirements for interpretation (point pairs above 1 % of the total number of point pairs) up to distance class ten and have therefore been interpreted for 2/3 of the total distance (Legendre and Fortin 1989, Rossi et al. 1992). For the omnidirectional correlograms, individual autocorrelation statistics are significant when tested against the null hypothesis that I does not differ from its expected value which is $-1/(n-1)$, where n is the number of fruit, according to the test procedure of Cliff and Ord (1981) (Legendre and Legendre 1998). Overall correlogram significance was tested using Bonferroni's correction for multiple comparisons (Legendre and Legendre 1998, Rice 1989).

All abundance and richness data were transformed ($\log_e (x+1)$) prior to analysis and correlogram construction (to stabilise the variance in the data), whereas environmental surface data (codes (0, 1) of fruit occurring in the sun or shade treatments) was not transformed (Dutilleul and Legendre 1993, Legendre and Legendre 1998). Only species occupying >30 fruit over the sampling period were used in autocorrelation analyses because this is an approximate minimum occurrence necessary (Sokal and Oden 1978, Legendre and Fortin 1989). Therefore, the abundances of *D. melanogaster, D. buzzatii, D. busckii* and *Zaprionus* msg 2 were not considered individually. Correlograms were thus constructed for *D. simulans*, *Zaprionus* msg 1 (not constructed for analysis of sampling occasion ten), the abundances of all species combined and species richness for the pooled data and sampling occasion 10. Therefore, the correlograms represent the quantified autocorrelation outputs of the environmental surface (experimental design) (Fig. 2.1) and biological responses surfaces (Fig. 2.2) and are referred to as I_E (environmental) and I_B (biological), respectively. Periodicity in a variable is defined here as repeated alternation across the correlogram of significantly positive or significantly negative I values.

[2] Sturge's rule is often used to decide on the number of distance classes in a histogram so that the resolution (more resolution with more distance classes) and power of the test are maximised.
Number of classes = $1 + 3.3\log_{10}$ (m), where m = the number of point pairs in the analysis

Comparison of biological outputs with environmental output

The discrepancy between the strengths of the environmental and biological outputs (for the omnidirectional correlograms) was expressed as a proportion of the environmental output (I_B/I_E). This discrepancy in output strengths between the biological and environmental outputs results from natural variation (I_N). Because Moran's I is bounded between +1.0 and –1.0 (Sokal and Oden 1978, Legendre and Legendre 1998), I_N was standardised across distance classes as 1-(I_B/I_E).

Cross-correlograms were used to compare the correlogram shapes of the biological output with the environmental output for the pooled data (Legendre and Legendre 1998). Cross-correlations were computed using the series of I values for distance classes 1--10 for each variable in the comparison. The lag at which the cross-correlation coefficient is greatest (and significant) represents the lag (distance) where the two series show the highest correspondence.

Although cross-correlations are useful for assessing the similarity or difference in spatial structure between two surfaces, they are spatially inexplicit within the study arena and are therefore unable to match the spatial structure of two surfaces to corresponding spatial localities within these surfaces (Perry et al. 2002). Therefore, spatially explicit analyses were performed using Spatial Analysis by Distance IndicEs (SADIE), to relate the biological response at an individual locality to the environmental surface at the same locality across sampling occasions (Dale et al. 2002, Perry et al. 1996, 2002, Perry 1998). SADIE explicitly incorporates spatial information associated with samples (localities) into the quantification of spatial pattern, and was used to determine the degree of aggregation in abundance (total and individual species) across the study arena, as well as to examine spatial aggregation at individual localities (fruit) (Perry 1995, 1998, Perry et al. 1996, 1999). SADIE is ideally suited to biological count data, such as abundance, which often contains zero values that skew the data distribution. SADIE assigns a sample an index of aggregation (I_a), and probability of aggregation (P_a), by comparing the spatial arrangement of the observed distance to regularity with the permuted distances to regularity derived from a randomisation procedure (Perry 1995). Values of I_a greater than 1.0 indicate spatial aggregation, those approximating 1.0 indicate randomness, and those less than 1.0 indicate regularity (Perry 1995).

SADIE is also able to identify patches (ν_i >1.5; areas of high abundance) and gaps (ν_j <-1.5; areas of low abundance) (Perry et al. 1999). The ν_i and ν_j values for each fruit for total abundance and the abundance of each species were plotted for visual inspection of clustering across the study arena (Perry et al. 1999). The average patch ($\bar{\nu}_i$) and gap ($\bar{\nu}_j$) distances were

calculated to formally test for overall clustering in total abundance and the abundance of each species across the study arena. Xu and Madden (2003) showed that the magnitude of SADIE patches and gaps are dependent on their distance from the centre of the study arena. However, the effect is unlikely when more than two patches are present (Xu and Madden 2003). Here, the study arena consists of six plots (three are likely to contribute to patches and three to gaps) and the arena is held constant throughout sampling. In addition, the centre of the arena lies on the boundary between a sun and shade plot, and because of the experimental layout (Fig. 2.1) additional sun and shade plots lie at equal distances on either side of the centre point. Furthermore, I_a, $\bar{\nu}_i$ and $\bar{\nu}_j$ values were not compared across studies, rather the presence of significant patches and gaps was determined and where these are located relative to the imposed microclimatic treatment plots. Therefore, the criticism raised by Xu and Madden (2003) is unlikely to bias the conclusions drawn from the SADIE results presented here (see also Veldtman and McGeoch 2004).

Literature overview

Studies examining spatial structure in population and assemblage parameters (including measures of population size, survival and mortality, species richness and total assemblage abundance) were extracted from the literature using the individual search terms 'Moran's *I*', 'correlogram' and 'spatial autocorrelation' in ISI Web of Science - Science Citation Index Expanded (http://isi10.isiknowledge.com/portal/cgi/wos). A total of 43 publications were found including 205 independent *I* values. Significant *I* values for only the first distance class were used. Non-significant *I* values may represent no spatial structure or too few point pairs to reach significance (Legendre and Legendre 1998). Furthermore, autocorrelation is most likely to be at a maximum in the first distance class (see e.g. Legendre and Fortin 1989). The lag distance in this class was used as a measure of the grain of the study (Bjornstad et al. 1999, McGeoch and Gaston 2002). Studies were divided into four grain categories: micro-grain (including studies with lag distance $\leq$ 1 m), local-grain (1m < lag distance $\leq$ 1 000 m), landscape-grain (1 000 m < lag distance $\leq$ 10 000 m) and regional-grain (lag distance > 10 000 m). The lag in the mesocosm used in this study was 0.27 m, and therefore falls within the micro-grain category.

Results

Quantification, confirmation and comparison of outputs with predicted output

The directional correlograms show that, as designed, the environmental surface (fruit coded for occurrence in sun or shade treatments) was autocorrelated across the study arena and correlogram structure varied with direction (Fig. 2.3a). Periodicity in the structure of the environmental surface was apparent for all directions and reflected plot structure in those directions across the experimental arena (Fig. 2.3a). The environmental surface was positively autocorrelated over the first four distance classes (x-intercept approximately in class four; approximately 6 fruit neighbours) and negatively autocorrelated from distance classes 5--10, reflecting plot structure in the N-S direction (0^0). The maximum negative autocorrelation between distance classes 6--10 resulted from single sun and shade plots in this direction (see Fig. 2.1). The periodicity in the 90^0 (W-E direction) correlogram (Fig. 2.3a) was a consequence of the start of the third sun or shade plot in that direction (Fig. 2.1). As predicted, for the remaining directions (45^0 and 135^0) plot structure was not revealed as clearly (Fig. 2.3a). Therefore, the patterns were not identical in the four directions. These periodic spatial patterns reflected plot structure of the environmental surface in the four directions. The directional correlograms for total fly abundance (one of the biological variables) (Fig. 2.3b, range of I in distance class 1 for total abundance: 0.380 to 0.518) reflected the same spatial pattern as their corresponding environmental correlograms (Fig. 2.3a).

Using omnidirectional correlograms, the environmental surface was also significantly autocorrelated across the study arena (Bonferroni α = 0.001; Fig. 2.4a). This surface was significantly positively autocorrelated in the first three distance classes (approximately 5 fruit neighbours) and classes 7--10 (approximately 10--14 fruit neighbours), and significantly negatively autocorrelated in classes 4--6 (approximately 6--9 fruit neighbours) (Fig. 2.4a). The x-intercept lay between distance classes 3--4 (or 5--6 fruit neighbours), approximating the length of one side of the square treatment plots. Thus the periodicity present in the autocorrelation profile reflected the combined spatial pattern of the environmental surface for all four directions. Nonetheless, the amplitude of the first peak was 0.79, i.e. lower than the maximum possible 1.0 and lower that the maximum value achieved using directional correlograms (range 0.63 to 0.82) (Fig. 2.4a).

The biological variables were also significantly autocorrelated across the study arena for the pooled data (Fig. 2.4b-e, I_B plots). The lag distance of peaks in the correlogram, i.e. significantly autocorrelated areas, in species richness, abundance and *D. simulans* abundance

approximated the lag distance of peaks in the environmental surface (x-intercept at or between distance classes 3--4, equivalent to 5--6 fruit) (Fig. 2.4a-d). Only *Zaprionus* msg 1 abundance was significantly positively autocorrelated over a smaller area than the other variables, i.e. 3 fruit, although the x-intercept also lay between classes 3--4 (Fig. 2.4e). The periodicity apparent in the environmental output was also present in total, *D. simulans* and *Zaprionus* msg 1 abundance, but not in species richness (Fig. 2.4). Distance 5 corresponds to the distance between adjacent sun and shade plots and all the biological variables were found to be less similar than expected by chance (negatively autocorrelated) over this distance (Fig. 2.4b-e). Therefore, the periodicity of the biological output was qualitatively similar to the environmental output.

The strength of the I_B values for sampling occasion 10 was weaker for species richness, total abundance and *D. simulans* abundance than for the pooled data (Fig. 2.4b-d). However, the form of the spatial output observed generally matched both the predicted output and the output observed for the pooled data (except that species richness was no longer significantly autocorrelated in the first distance class, Fig. 2.4b). Therefore, the removal of the spatiotemporal mechanism did not affect pattern detection but did weaken the output by 54 to 74 % across the first 3 distance classes relative to the correlograms of the pooled data (see Fig. 2.4b-d).

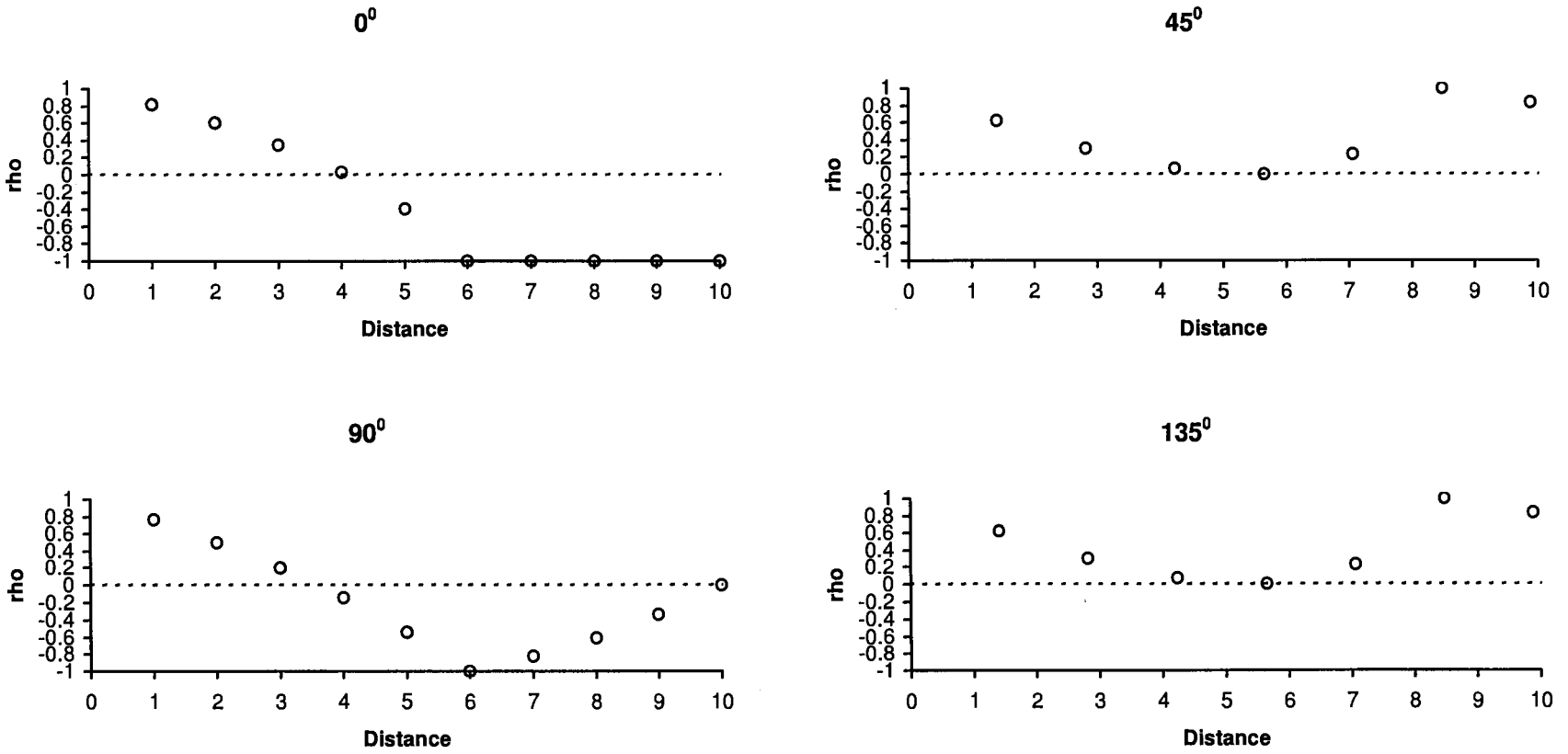


Fig. 2.3a. Directional correlograms (standardised covariograms) for the environmental surface as determined by the experimental design. Rho = ratio of standardised covariances (Kaluzny et al. 1998). See figure 1 for explanation of directions.

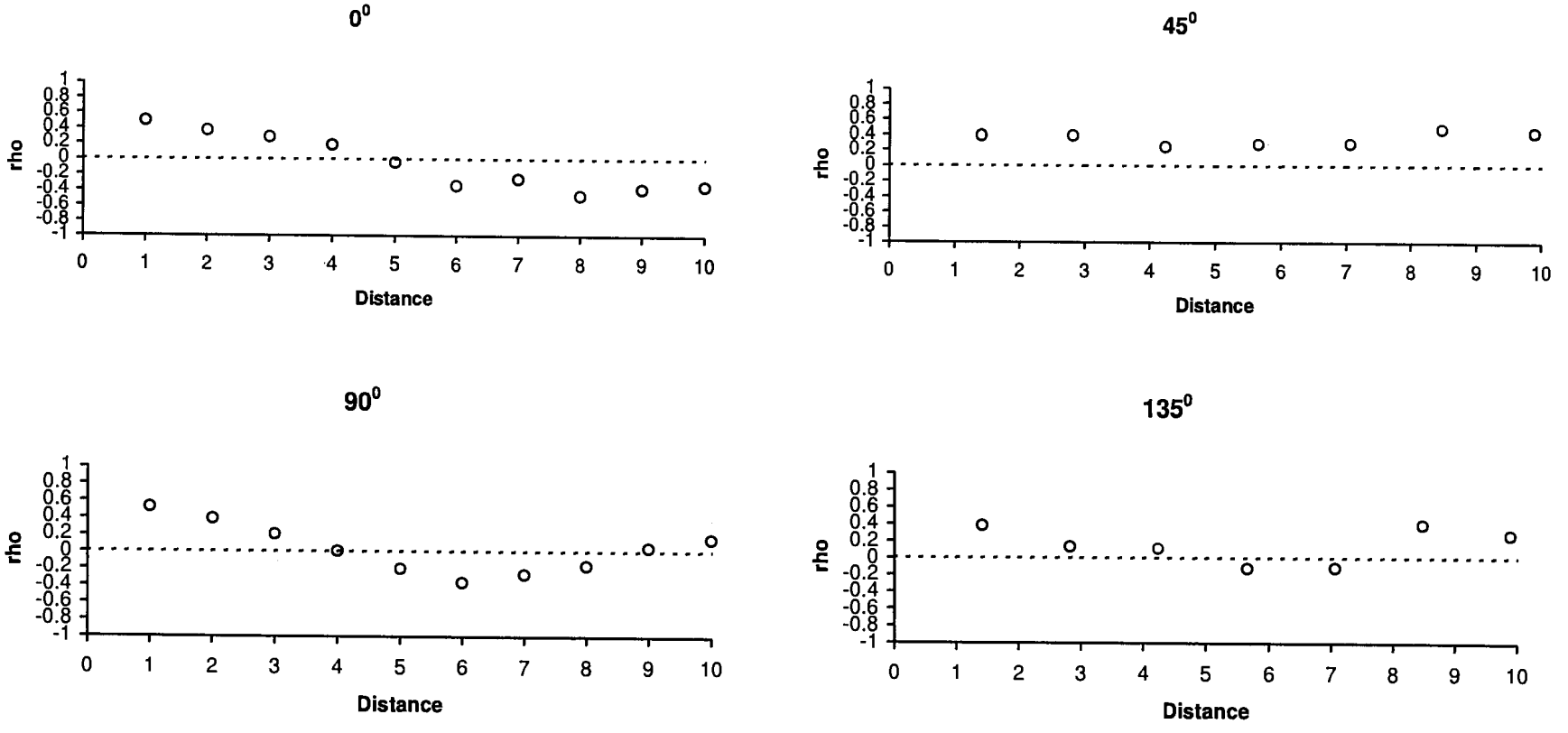
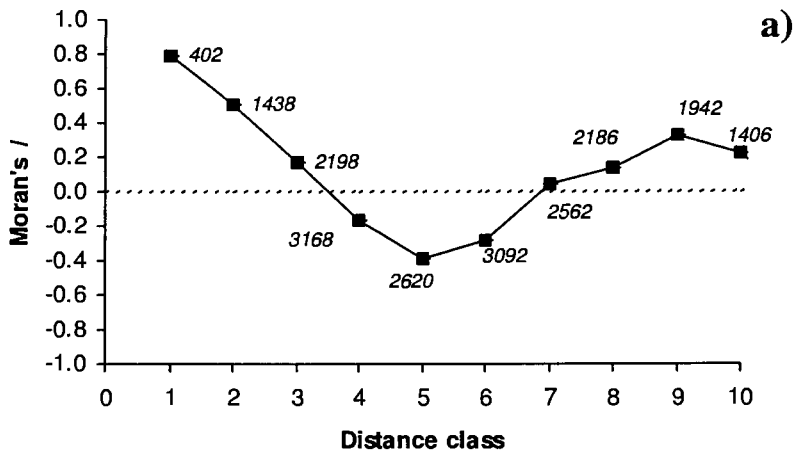
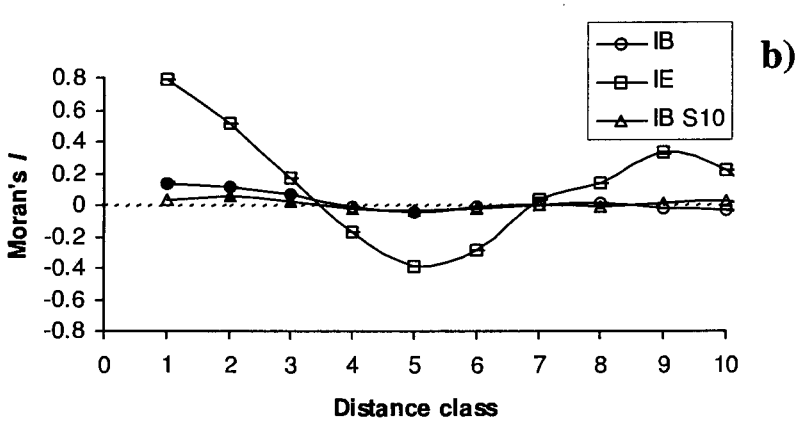
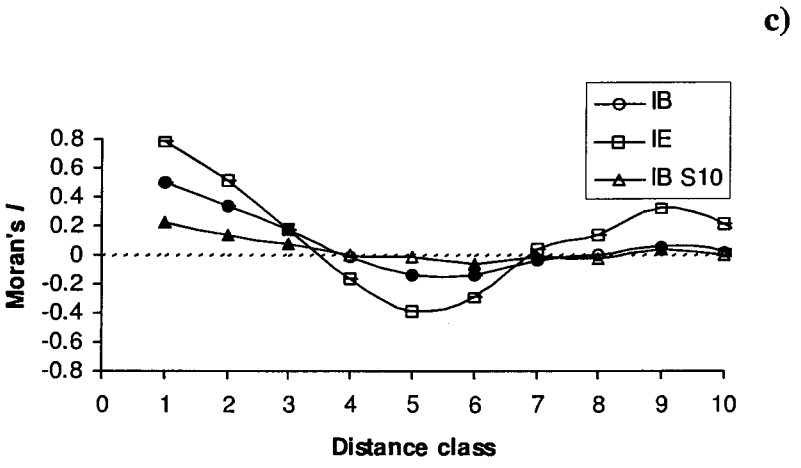


Fig. 2.3b. Directional correlograms (standardised covariograms) for total abundance of all species. Rho = ratio of standardised covariances (Kaluzny et al. 1998). See figure 1 for explanation of directions


a)
Moran's I
Distance class
402
1438
2198
3168
2620
3092
2562
2186
1942
1406


b)
IB
IE
IB S10
Moran's I
Distance class


c)
IB
IE
IB S10
Moran's I
Distance class

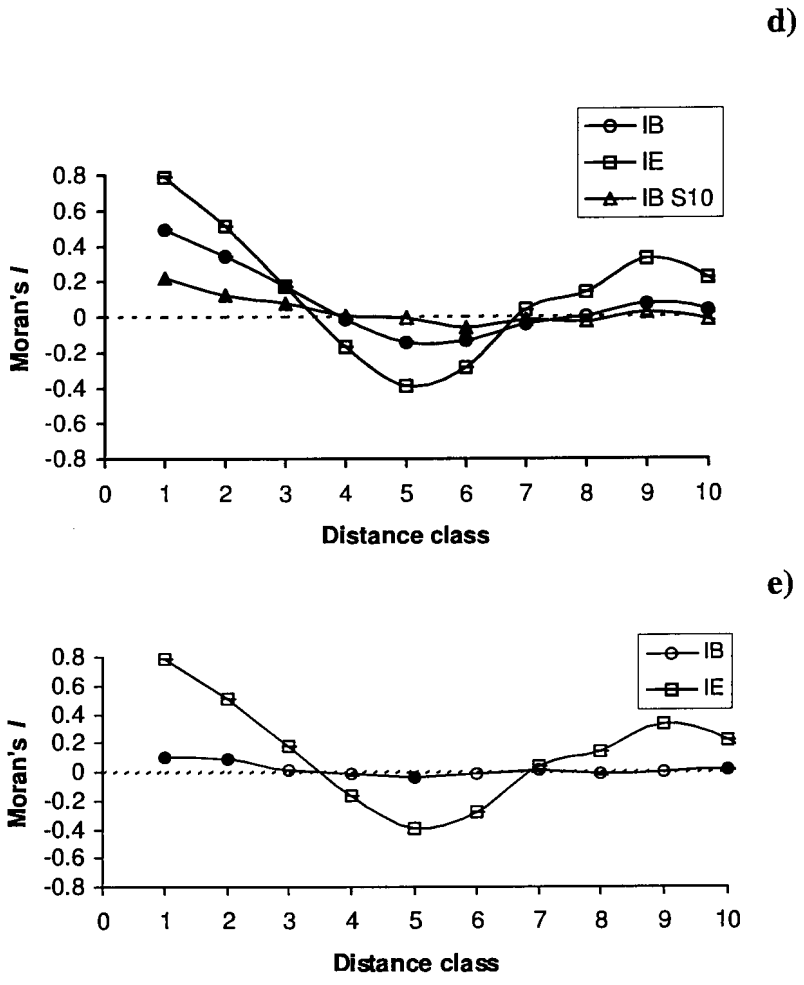


Fig. 2.4. Autocorrelation output (omnidirectional correlograms of Moran's *I*) for a) the environmental surface as determined by the experimental design (I_E), b) species richness, c) total abundance, d) *D. simulans* abundance and e) *Zaprionus* morphospecies group 1 abundance (I_B and I_B S10 for the pooled data and sampling occasion 10 respectively, in each case). Closed points represent significant Moran's *I* values at $P < 0.05$. The number of point pairs in each distance class appears in italics for a) the environmental surface, and are the same throughout for the pooled data. Bonferroni corrected overall correlogram significance levels as follows: a-d: $\alpha = 0.001$ and e: $\alpha = 0.01$. For a-e, error bars are +/- one S.E. of the individual points (too small to be visible in most cases). The difference in *I* between I_E and I_B is attributable to natural variation (see text for details).

Comparison of biological outputs with environmental output

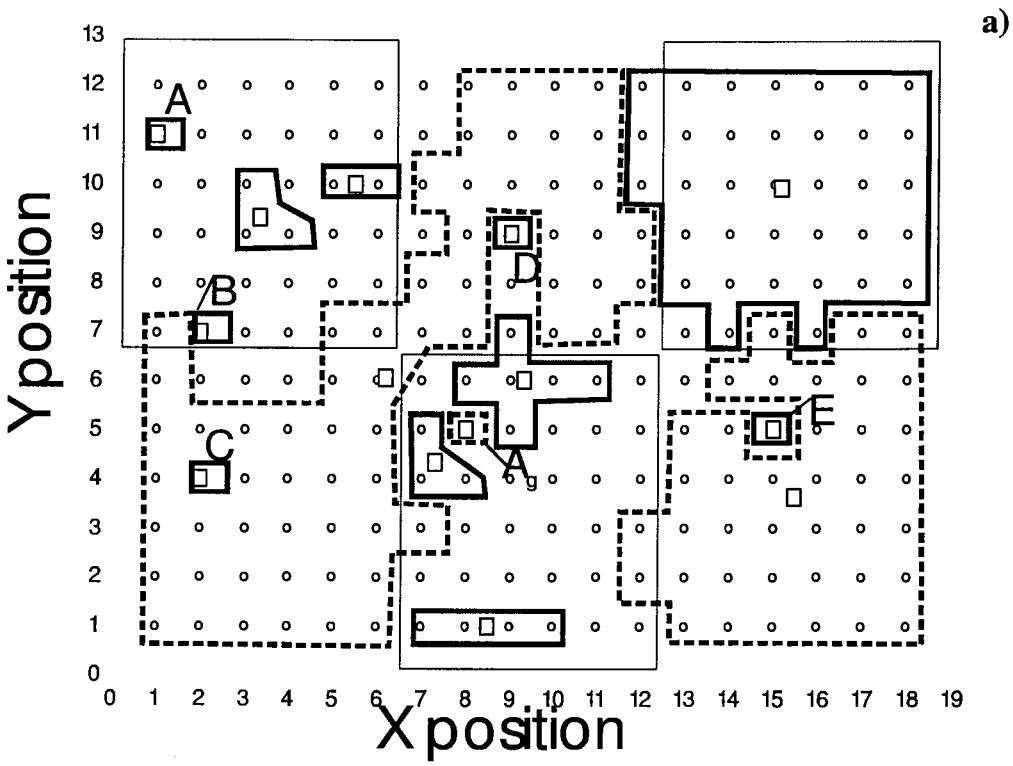
The qualitative similarity in the biological and environmental outputs was supported by cross-correlation analyses. Individual cross-correlograms for the environmental surface (I_E) and the respective autocorrelation outputs of the biological response surfaces (Moran's I_B values) were all significantly positively correlated at a lag of zero (Table 2.1). No significant correlations were found beyond this lag distance. The pairs of series' thus corresponded most closely with no spatial lag. Therefore, the shape of the spatial pattern that was detected for the biological response surfaces using autocorrelation analysis largely corresponded with the spatial output of the environmental surface, or microclimate plot structure (Fig. 2.1).

The SADIE output confirmed what was predicted for the biological output, namely, that patches and gaps coincided with shade and sun plots respectively. The patch ($\bar{v}_i$) and gap ($\bar{v}_j$) positions for total, *D. simulans* and *Zaprionus* msg 1 abundance, in general, coincided with the shaded (patch positions) and exposed (gap positions) plots of the experimental arena (Fig. 2.1, Fig. 2.5a-c). Three gaps encompassing 100 fruit (dashed lines) and eleven patches encompassing 58 fruit (thin solid lines) were identified for total abundance (Table 2.2, Fig. 2.5a). One of the gaps and five of the patches consisted of only a single fruit (gap A_g, patches A-E, Fig. 2.5a). The remaining two gaps were larger than any patches, i.e. the size of clusters of low abundance counts were larger than clusters of high abundance counts.

Although the distribution of individuals within species tended towards statistical aggregation (variance greater than the mean), only *D. simulans* and *Zaprionus* msg 1 were significantly aggregated into patches and gaps (Table 2.2, Fig. 2.5b,c). Similar to the spatial structuring of total abundance, fewer gaps than patches were identified for individual species and these were always larger than the patches (Table 2.2, Fig 2.5b,c). No patches were identified for *D. melanogaster* and *D. buzzatii* (Table 2.2). The formation of patches and gaps for the individual species also corresponded to the experimental plots of the study arena that were shaded and in the sun respectively (Fig. 2.5b,c), although one large gap for *Zaprionus* msg 1 extended into a shaded plot (Fig. 2.5c). The use of the spatially explicit SADIE method thus demonstrated that areas of high and low numbers of flies coincided physically with shade and sun plots respectively. Furthermore, the sizes of patches, with the exception of one *D. simulans* patch, were small, comprising between one to eight fruit supporting the expectation of within and across fruit oviposition patterns (Fig. 2.5).

While the shapes of the environmental and biological autocorrelation outputs were similar, the strengths of the omnidirectional biological outputs were weaker than those of the

environmental output. The amplitudes of the first peak and trough were lower for all biological outputs than the environmental output (Fig. 2.4). The amplitudes of the first peak and trough (pooled data) for total abundance and *D. simulans* were strongest (I = 0.49 to I = -0.29), while the amplitudes of species richness and *Zaprionus* msg 1 were weaker (I = 0.14 to -0.34 and I = 0.10 to -0.05 respectively) (Fig. 2.4b-e). Graphically, the strengths of the biological outputs were indeed mostly weaker than the environmental output across the entire correlogram (Fig. 2.4, 2.6). This difference in the quantified biological outputs (I_B) and the environmental output (I_E) is the autocorrelation in the biological variable that may be attributable to natural variation (I_N). For example, I_N weakened the output of species richness by 10-62 %, while total abundance I values were weakened by 33-99 % for the first 10 distance classes (Fig. 2.6a). For the first distance class, I_N values were 0.82 and 0.38 for species richness and abundance and 0.87 and 0.38 for *Zaprionus* msg 1 and *D. simulans* (Fig. 2.6). Therefore, the biological output was, in general, always weaker and sometimes substantially so, than the output of the environmental surface for a specified distance class.



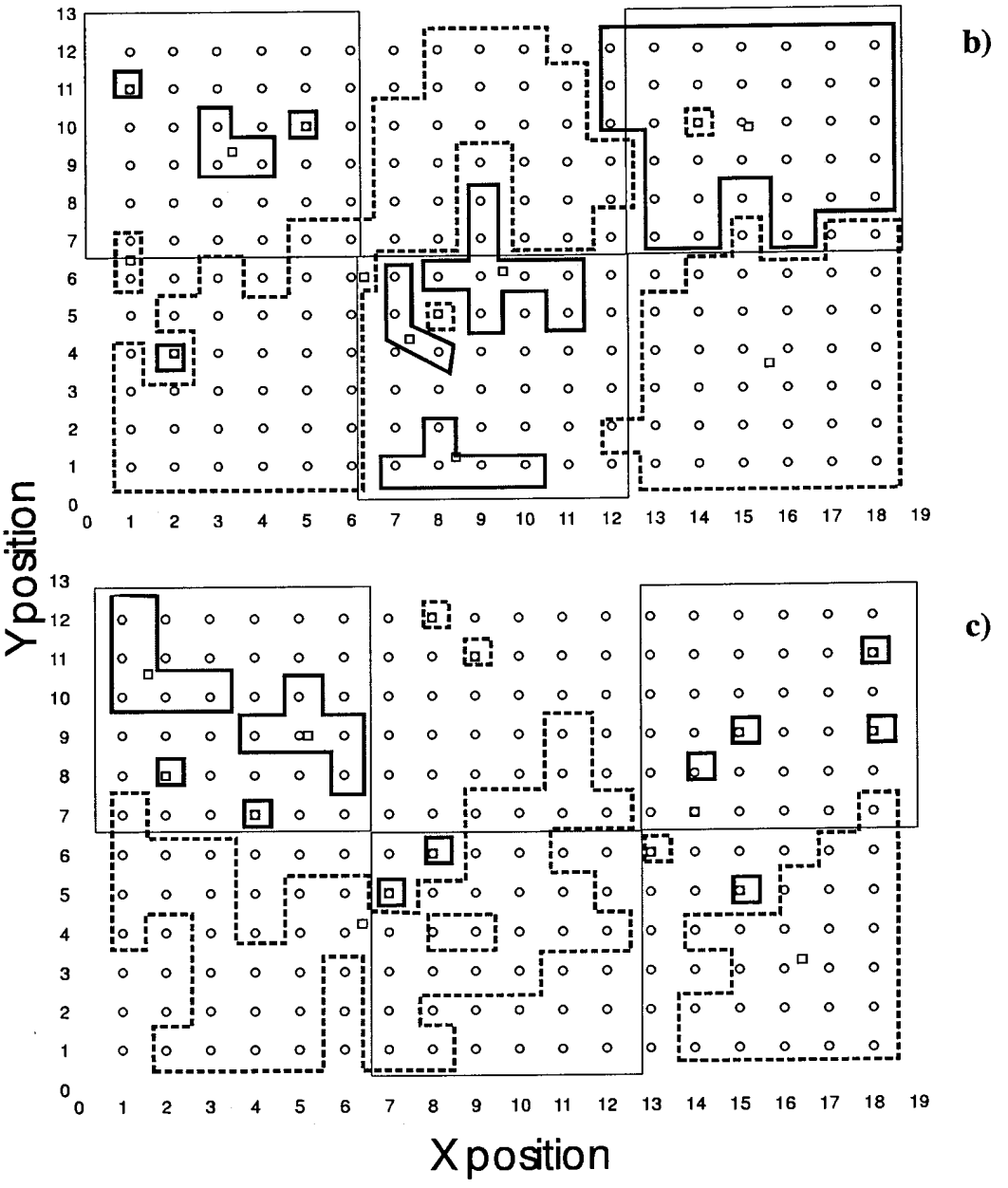


Fig. 2.5. Spatial positions of gaps (dotted outline; v_j < -1.5) and patches (thick solid outline, v_i > 1.5) for a) total abundance, b) *D. simulans* and c) *Zaprionus* msg 1. Circles represent individual fruit. Large squares represent centroids of gaps and patches. Thin solid square outline around groups of circles represent fruit that were shaded by 80 % shade netting (as in Fig. 2.1), with remainder representing fruit exposed to the sun. A_g = single fruit constituting an abundance gap; B,C, D, and E = single fruit constituting an abundance patch.

Table 2.2. Aggregation indices and the number and size (represented by number of fruit) of gap and patch clusters. I_a = Index of patchiness, $\bar{v}_j$ = the average value of v_j over all inflows, $\bar{v}_i$ = the average value of v_i over all outflows, gap = area of low abundance, patch = area of high abundance. * = P < 0.05, *** = P < 0.001 (Dsim= *Drosophila simulans*; Zap1 = *Zaprionus* morphospecies group 1; Dmel = *D. melanogaster*; Zap2 = *Zaprionus* morphospecies group 2; Dbuzz = *D. buzzatii*; Dbusck = *D. busckii*).

Aggregation index	Total abundance	Dsim	Zap1	Dmel[1]	Zap2[1]	Dbuzz[1]	Dbusk[1]
mean; variance	36.64; 2159.46	35.27; 2077.08	0.70; 3.28	0.20; 0.54	0.23; 0.52	0.09; 0.79	0.06; 0.12
Index of dispersion	12670.30	12662.10	1009.11	564.73	493.20	1837.60	456.00
I_a	2.33***	2.33***	1.51	0.92	1.24	0.87	1.06
$\bar{v}_j$	-2.22***	-2.26***	-1.51*	-0.91	-1.25	-0.87	-1.04
$\bar{v}_i$	2.58***	2.49***	1.42*	0.94	1.33*	0.97	1.15
Clusters							
(number, size)							
gap	3, 100	5, 100	5, 74	9, 25	8, 54	2, 26	1, 39
patch	11, 58	8, 56	11,19	0, 0	5, 8	0, 0	1, 1

[1]SADIE results may suffer from high Type II error rates i.e. lack of power because of low occupancy of these species (Korie et al. 2000).

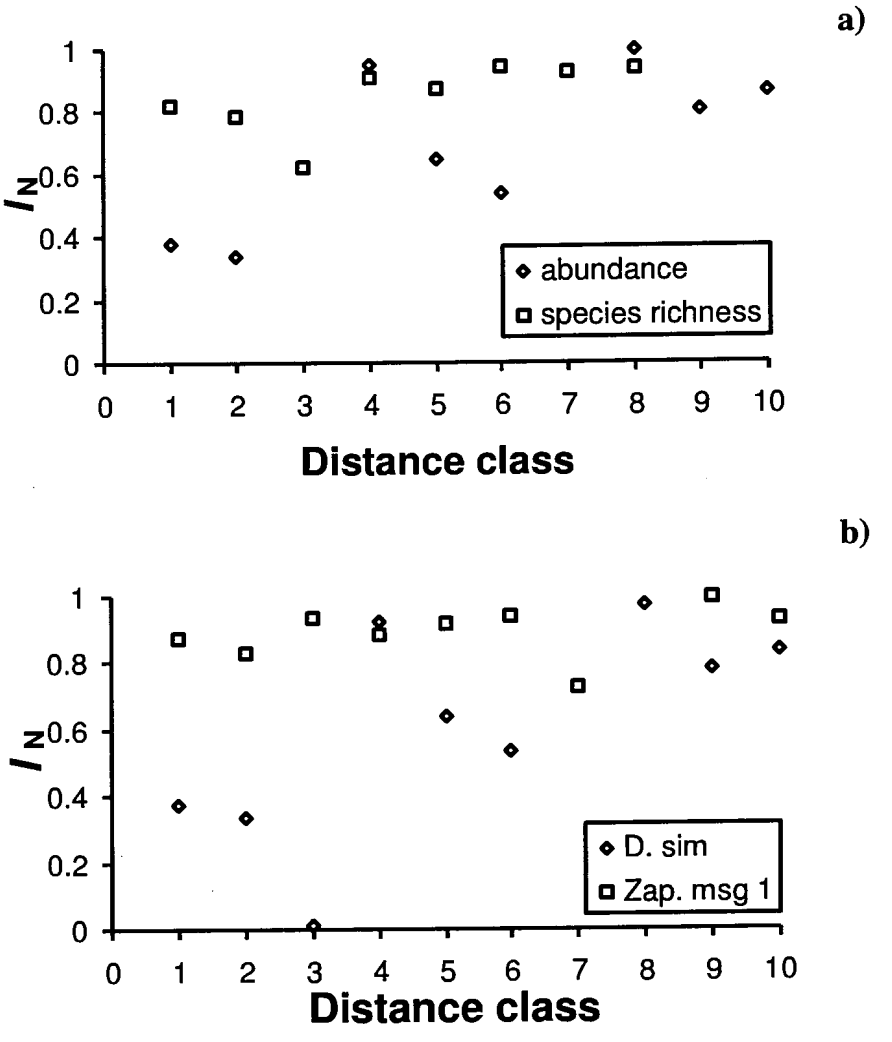


Fig. 2.6. Strength of dampening by natural variation (I_N) on a) assemblage variables and b) individual species abundances (D. sim = *D. simulans* and Zap. msg 1 = *Zaprionus* msg 1) *I*-values. Proportions that tend towards positive 1.0 represent distance classes where the strength of the dampening by I_N on the biological output Moran's *I* is maximised; missing values for distance classes represent classes where the biological and environmental *I* values were in the opposite direction (viz. positive and negative autocorrelation) and it would therefore be nonsensical to interpret the size of the output. $I_N = 1 - (I_B/I_E)$.

Weak spatial pattern

As expected, I_B of the rare species (*Zaprionus* msg 1, I values ranged from 0.003 to 0.50) was always weaker than the most abundant species (*D. simulans*, output ranged from 0.08 to 1.8) across distance classes (Figs 2.4d,e). Nonetheless, the autocorrelation coefficient of the rare species was positive, albeit small, for distance class three (five fruit neighbours), reflecting plot structure at this distance (Fig. 2.4e). Therefore, the spatial structure in the rare species was biologically meaningful because it matched the environmental surface (higher abundances in shade than sun, see also Fig. 2.5c), although not all I_B values were statistically significant (Fig. 2.4e). However, some of the individual I_B values that showed a weak output (small I_B) for *Zaprionus* msg 1 (e.g. I = 0.02 to –0.05 beyond class 3) were both statistically significant and biologically meaningful (Fig. 2.4e). The periodicity that was observable for the autocorrelation profile of the rare species also corresponded to the distances over which autocorrelation was expected to be positive or negative in the environmental surface. Consequently, although the spatial output (I_B) for the rare species was substantially weaker than the common species, periodicity was still detectable and corresponded to the environmental surface.

***I* values in the literature**

There was a general shift in the mode of I value distributions towards greater distance classes with an increase in the grain of the first distance class across the 43 studies examined, particularly between micro-, local- and landscape-grain studies (Fig. 2.7). At the micro-grain the mode was 0.1, at the local-grain 0.3, at the landscape-grain between 0.6 and 0.7 (Fig. 2.7). At the regional-grain the mode was at 0.4, with a strong peak at 0.6 (Fig. 2.7). The percentage of studies with I values of > 0.5 was greater in landscape- than in micro- and local-grain studies (Fig. 2.7). No studies were reported with maximised I values (I = 1.0, Fig. 2.7).

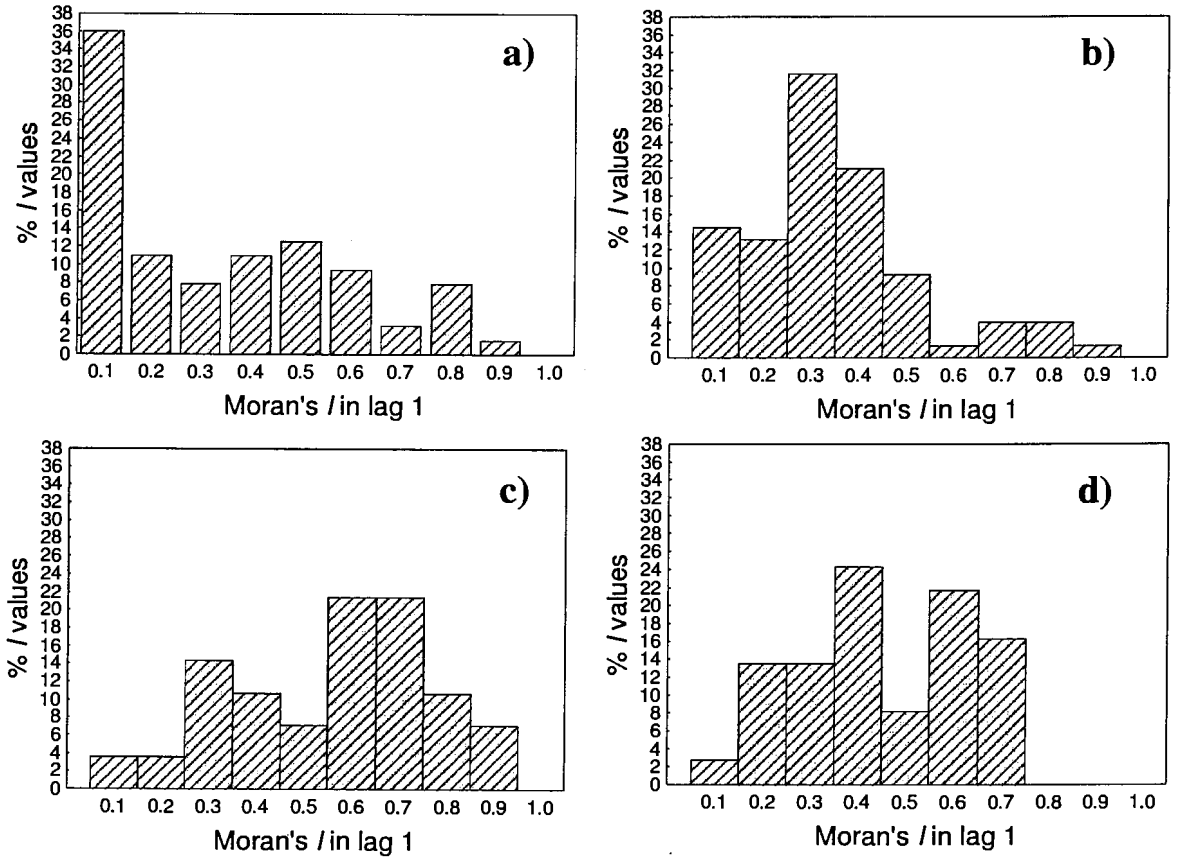


Fig. 2.7. Percentage of significant Moran's *I* values of between 0.1 and 1.0 in the first distance class, in a) micro-grain (9 studies, 64 *I* values), b) local-grain (13 studies, 76 *I* values), c) landscape-grain (6 studies, 28 *I* values) and d) regional-grain (16 studies, 37 *I* values) studies.

Discussion

The mesocosm employed here generated a level of biological complexity in the drosophilid assemblage that is absent from most simulated studies as natural variability was included. The uncertainty of the potential causes of spatial structuring was reduced because structure was imposed *a priori* by the sun/shade treatments and drosophilids are known to respond to temperature variations (e.g. Feder and Krebs 1998, Worthen and Haney 1999). Abundance was much higher in two of the three shade plots, while the response of species richness to the imposed treatment across the study arena was not as clear as for abundance. Nonetheless, the assemblage did respond to shade and sun, with higher abundance and species richness in the shade than the sun plots. Spatial structuring (aggregation) within individual fruit and across adjacent fruit (a consequence of fly oviposition behaviour) (Feder et al. 1997 a,b, Heard and Remer 1997, Feder and Krebs 1998, Remer and Heard 1998, Worthen and Haney 1999, Warren et al. 2003) was also observed. Furthermore, the common and rare species responded in a similar manner, with highest abundances in the shade plots.

The results from the mesocosm therefore show for an empirical data set that spatial pattern in population and assemblage variables are effectively detected as such by spatial analytical procedures, and that the form of spatial structure in these variables accurately reflected that of the environmental surface. However, the strength of the biological response was weak, being dampened by 10-99 % across distance classes. Therefore, even for a comparatively simple empirical scenario, in this case one generated to control a large proportion of environmental variability, the spatial structure in the biological response was weak ($I < 0.3$).

While the strength and significance of these weak values may be affected by the number of point pairs included in their calculation (Koenig 1999, Legendre and Legendre 1998, Tobin 2004), these results show that such values should not be dismissed during the interpretation of correlograms. Similarly, Tobin (2004) demonstrated that although the strength of local (distance class 1) autocorrelation decreased with reduced sample size, the form of spatial structure (x-intercept) remained unaffected by sample size. His suggestion of how to overcome the error in estimating local autocorrelation is to increase sampling at shorter distances (Tobin 2004). However, as demonstrated here significant, weak responses do not necessarily result from the effect of reduced sample size on autocorrelation coefficient strength (for example, Tobin 2004) or the effect of large sample sizes inflating the significance of coefficients (for example, Koenig 1999). Therefore, weak spatial outputs in empirical population and assemblage variables may provide a valuable basis for hypothesis generation.

The review of published *I* values shows that in fully mensurate data sets, except at a landscape-grain, the distribution of *I* values in the first distance class is right-skewed. Therefore, spatial structure within the range 0.1-0.3 may be expected in empirical studies. Indeed, 55 % of the *I* values obtained from other studies at fine (micro-grain) spatial scales fell within the range 0.1-0.3, with the modal *I* value at 0.1. The maximum *I* values achieved for any biological variable in this mesocosm, i.e. total abundance, falls outside of the range 0.1-0.5 where > 75 % of *I* values achieved in fine scale studies in the literature lie.

However, when data are pooled (as they were in this study by pooling the data for sampling occasions one to eleven) another component of natural variation is added to the data. This equates to the inclusion of a spatiotemporal mechanism when data are pooled (Ives and Klopfer 1997). The *I* values for species richness, total abundance and *D. simulans* abundance for a single sampling occasion (i.e. sampling occasion 10) were weaker (by 0.5 to 0.75) than for the pooled data, suggesting that pooling samples over time (i.e. including the spatiotemporal mechanism of natural variation) strengthens the detected output. Nonetheless, pooling samples over time from the same locations did not contribute to the detection of spatial autocorrelation *per se*, as suggested by Ives and Klopfer (1997), as the unpooled quantified outputs (I_B S10) also matched the environmental surface output (I_E). Therefore, pooling samples over time is unlikely to affect the ability of spatial analyses to generate hypotheses as the form of spatial pattern is unaffected.

In addition to natural variability affecting the maximum strength of spatial output likely to be achieved, the spatial analytical procedure used may be responsible for some of the loss of signal strength. Omnidirectional correlograms combine the spatial pattern from multiple directions into one correlogram (Radeloff et al. 2000). Pooling different strengths of structures from multiple directions (when no anistropy is evident) results in averaged autocorrelation values in omnidirectional correlograms. A reduction of 4-5 % from the maximum *I* value was found when comparing autocorrelation values of directional correlograms with the corresponding omnidirectional correlogram in this mesocosm study (Environmental surface directional correlogram *I* value range class 1: 0.63 to 0.82, omnidirectional $I = 0.79$; Total abundance directional correlogram *I* value range class 1: 0.38 to 0.52, omnidirectional $I = 0.49$). Although this result is to be expected (indeed Radeloff et al. (2000) recommend the use of directional correlograms for checkerboard designs to prevent the loss of resolution), the large number of sampling points necessary to test for significance in directional correlograms (Dungan et al. 2002) may limit their usefulness in natural landscapes. Sampling point density is

often lower in these landscapes than for simulated studies (e.g. Radeloff et al. 2000, McGeoch and Price 2004; but see Dieleman and Mortensen 1999). However, the computation of directional correlograms is essential when anisotropic spatial structure is present (Legendre and Legendre 1998). If geometric anisotropy is common in nature, directional correlograms would remain more appropriate than omnidirectional correlograms (Radeloff et al. 2000, Perry et al. 2002, but see Rosenberg (2004) for new method using wavelet analysis).

This study provides an empirical demonstration in support of the several previous simulated studies demonstrating the value of spatial analytical procedures (see Legendre and Fortin 1989, Radeloff et al. 2000). After imposing environmental spatial structure in a mesocosm, the emerging biological patterns were used to examine the efficacy of spatial analytical procedures for detecting pattern in empirical data. Spatial pattern may be expected to be weak in micro-grain empirical studies. Published *I* values show that at fine (micro-grain) spatial scales the expected strength of the spatial output is indeed moderate to weak. However, these moderate to weak outputs are biologically realistic and weak pattern should therefore not be negated when observed in ecological studies, especially at finer spatial scales. Because spatial analysis is able to accurately detect weak spatial output, hypothesis generation will not be affected when spatial pattern is weakened substantially by natural variation in biological systems. What remains to be accomplished is to maximise the hypothesis generating capacity of spatial analysis.

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CHAPTER 3

A sensitivity analysis approach to improving hypothesis generation in spatial analysis

Introduction

Spatial analysis is a useful tool to investigate the variation in, and the mechanisms structuring, patterns in species richness and abundance across space. The development of this tool has lead to significant advances in the understanding of species distributions, as well as their abundance structures at particular localities (e.g. Cappuccino 1988, Brewer and Gaston 2002, 2003, Perry et al. 2002, Gaston et al. 2004, McGeoch and Price 2004). The factors likely to influence both the choice of spatial method and the output obtained in such studies have been thoroughly reviewed in the recent literature (Dale et al. 2002, Dungan et al. 2002, Keitt et al. 2002, Perry et al. 2002, Xu and Madden 2003). Some understanding has also been gained of the implications of the inclusion of natural variation for the interpretation of patterns in empirical data (see Chapter 2, Ives and Klopfer 1997). However, current understanding of how useful different techniques are for generating hypotheses is limited (Borcard and Legendre 2002, Dale et al. 2002, Perry et al. 2002). Therefore, whether it is possible to improve the capacity of spatial techniques to generate hypotheses requires further exploration.

Spatial analysis is generally viewed as a hypothesis-generating tool. Potential mechanisms affecting pattern may be identified and, thereafter, tested (Liebhold and Gurevitch 2002, Perry et al. 2002, McGeoch and Price 2004). In empirical data, three main categories of structuring mechanisms may be expected to generate quantified spatial output, i.e. to be the main sources of variation in the spatial output. These include extrinsic mechanisms (abiotic factors such as climate), intrinsic mechanisms (generated by the study organism, e.g. dispersal and aggregation behaviour) and natural variation across individuals in their response to different mechanisms (Brown et al. 1995, Logerwell et al. 1998, Radeloff et al. 2000, Austin 2002, Elith et al. 2002, Chapter 2). For example, within local habitat patches, spatial structuring in species richness, abundance and distribution may reflect behavioural (Cappuccino 1988), dispersal (Bach 1981, Duelli 1990, French et al. 2001), predation (Winder et al. 2001), resource quality (Bach 1981, Brown 1984, Peng and Brewer 1994, Summerville and Crist 2001), or microclimate (Levings 1983, Retana and Cerdá 2000) differences between patches. In addition,

individuals of a species may respond differently over time and space to the environment in which they find themselves, i.e. natural variation across individuals in their response to different mechanisms is present (*sensu* Elith et al. 2002) (Tobin and Pitts 2002, Papadopoulos et al. 2003). Spatial analysis is one means to distinguish between these multiple, potential categories of mechanisms.

To enhance the hypothesis generating capacity of spatial analysis multiple spatial analytical procedures may be used (Wiens 2000, Dale et al. 2002, Perry et al. 2002). Multiple techniques not only confirm hypotheses pertaining to the mechanisms effecting spatial pattern in biological variables but also generate additional hypotheses (see, for example, Perry et al. 2002). Nonetheless, the presence of a particular spatial pattern does not mean that the main, or all, mechanisms generating that pattern have been identified (Legendre and Fortin 1989, Diniz-Filho et al. 2003). If the magnitude of total spatial structure and the contribution of each potential mechanism to the detected structure are known, then the proportion of unexplained spatial variation may be estimated. A large proportion of unexplained variation means that at least some explanatory mechanism structuring the biological variable remains to be identified (Legendre and Legendre 1998). Therefore, determining the contribution of different mechanisms to total spatial structure will improve hypothesis generation. Such an assessment of the contribution of different structuring mechanisms to total spatial structure requires the partitioning of spatial structure into its constituent components (e.g. Borcard et al. 1992, Van Rensburg et al. 2002, Heikkinen et al. 2004, Titeux et al. 2004).

By decomposing the spatial structure in a variable into the amount of variation attributed to different categories of likely spatially acting mechanisms, it is potentially possible to assess the relative importance of mechanisms in generating the pattern observed (see for example, Borcard et al. 1992, Legendre and Legendre 1998, Elston et al. 2001, Engen et al. 2002). This, in the broadest sense, is a form of sensitivity analysis, where the variation in the output of a model (in this case a geostatistical, e.g. autocorrelation, model) is apportioned, graphically or statistically, to different sources of variation (Chan et al. 1997, Saltelli et al. 1999, Saltelli 2002). Therefore, hypothesis generation may be improved through the additional partitioning of the spatial component of biological variation to different spatial mechanisms.

In this study, the efficacy of a sensitivity analysis method, developed here, to improve the hypothesis generating capacity of spatial analysis is examined. The ability of the sensitivity

analysis method to distinguish between different categories of mechanisms and to assess the strength of these mechanism categories in determining spatial structure is evaluated. This is achieved through the use of multiple graphical methods and the partitioning of spatial variation into categories of mechanisms. As an in depth understanding of the system is often required to interpret spatial pattern at multiple scales (Borcard et al. 2004), a mesocosm was used to provide a platform for both the generation and testing of hypotheses (see also Chapter 2, Englund and Cooper 2003, Warren et al. 2003). The mesocosm generated a heterogeneous environmental surface and this environment was colonised naturally by a assemblage of vinegar flies (Diptera: Drosophilidae). A previous study has demonstrated that the flies respond differentially to the imposed sun and shade treatments, generating spatial patterns in the abundance structures of the species utilising this mesocosm (Chapter 2).

Materials and Methods

Study organisms and sampling design

An urban Drosophilidae assemblage that inhabits decaying fruit (in this case nectarines (*Prunus persicae* Miller: Rosaceae)) was used in a mesocosm consisting of 216 similarly sized fruit that were washed and weighed at the start of the experiment (Warren et al. 2003). The variation in fruit mass was small and not significantly different between sun (Mean ± S.E. = 58.59 g ± 1.09) and shade (Mean ± S.E. = 59.08 g ± 1.07) plots ($t = 0.35$, $df = 214$, $P = 0.72$). Therefore, resource availability (fruit mass) was assumed to play no role in explaining assemblage differences between treatments. Five additional nectarines were randomly selected for insecticide residue tests and were found to have no detectable levels of residues of the following chemicals used in the local soft fruit industry: organophosphates (Dimethonate, Malathion, Triazophos), organochlorides (γ-BHC, β-Endosulphan, Endosulphan sulphate) and pyrethroids (Cypermethrin, Deltamethrin) (South African Bureau of Standards).

A wire table (2.4 m x 3.6 m; 0.7m high) was placed out at the University of Pretoria's Experimental Farm in Pretoria, South Africa (25°45.178"S, 28°15.293"E). The table was located inside a cage covered with pigeon wire to exclude birds, fruit-piercing moths and large wasps, while allowing the flies ready access to the fruit. Ants were also excluded by the application of grease to the table legs. The table (hereafter the 'study arena') was divided into six equal plots with each plot supporting 36 nectarines (Fig. 3.1). The mesocosm, albeit at a smaller scale, was

thus typical of the layout and symmetry found in agricultural ecosystems and characterised by few patches of a single type (Perry et al. 2002). Three of the plots were artificially shaded with 80 % shade netting to impose variation in microclimate across the study arena, and thereby add a level of spatial heterogeneity in microclimate to the experiment (Fig. 3.1). Six copper-constantan thermocouples were placed 1 cm under the top skin of six nectarines (three fruit were exposed to the sun and three were shaded) to measure the temperature of the fruit every 10 minutes for the duration of the experiment (recorded by a Campbell Scientific CR10 datalogger using PC208 software for programming and data capture). The experiment ran from 6 November to 1 December 1998.

The same dataset as has been used in Chapter 2 is used here. Chapter 2 examines pattern detection and compares the strength of autocorrelation at a micro-grain scale with published literature. Chapter 3 examines improving our understanding of the mechanism categories generating pattern. Using the same data set in no way complicates these two goals as the two chapters deal with different aspects of spatial structure. That said, a good test of the sensitivity analysis approach that is developed here (see below) would be to use a replicated study of the one already used in Chapters 2 and 3. Although the study was repeated, this data was not used.

The nectarines were placed 20 cm apart in a regular grid of 12 x 18 fruit. Each fruit was placed on coarse plastic mesh in the centre of a round plastic container (~ 15 cm diameter and 8 cm deep) containing washed, moist sand. A fixed volume of water was sprayed into the containers every day to ensure that the sand remained moist. *Drosophila* larvae drop down into the substrate under the fruit and pupate at 4.5 to 8.5 days for flies at 25 °C and 80 % RH (Sevenster and Van Alphen 1993). Larvae were therefore allowed to pupate in the sand underneath the fruit before being collected in plastic jars and being taken to the laboratory. Starting from the fifth day after exposure of the fruit, the sand containing the fly pupae under the nectarines was removed and placed in 350 ml plastic jars every second day for 25 days (11 sampling occasions). Fresh, moist sand was immediately placed in the containers under the fruit at each collection. The plastic jars were taken to the laboratory and the emerging flies identified according to McEvey et al. (1988). At least six Drosophilidae species were found, however, specimens belonging to the genus *Zaprionus* could not be identified to species level. The identified species were: *Drosophila simulans* Sturtevant, *D. melanogaster* Meigen, *D. busckii* Coquillett, *D. buzzatii* Patterson and Wheeler, *Zaprionus* morphospecies group 1 (*Zaprionus*

msg 1) (may include both *Z. tuberculatus* Malloch and *Z. sepsoides* Duda) and *Zaprionus* morphospecies group 2 (*Zaprionus* msg 2) (may include both *Z. vittiger* Coquillett and *Z. indianus* Gupta) (McEvey et al. 1988).

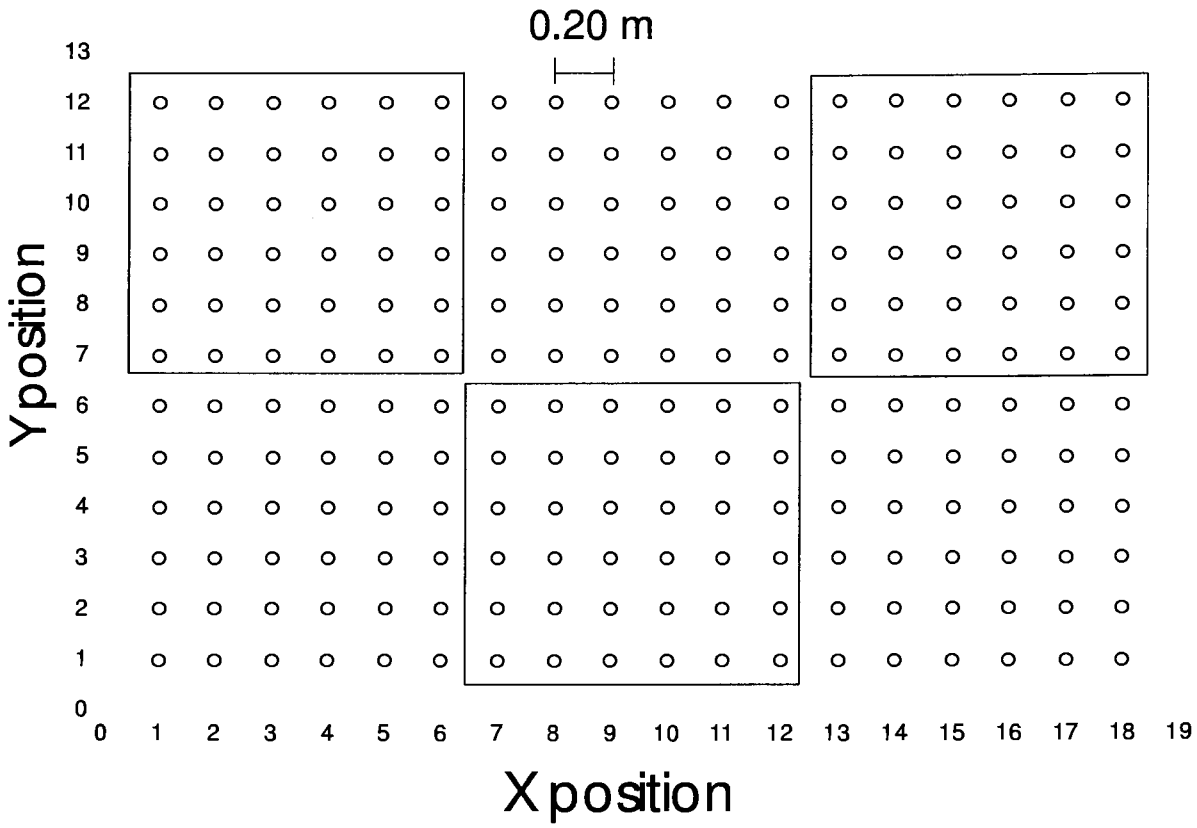


Fig. 3.1. Experimental plot (study arena) depicting the layout of decaying fruit (X position = 3.6 m x Y position = 2.4 m). Thin, solid rectangular outline around groups of fruit represent plots that were shaded with 80 % shade netting. Unblocked circles represent fruit exposed to the sun. Each plot contained 36 nectarines spaced 20 cm apart in a regular grid.

Sensitivity analysis

Following the broad definition of sensitivity analysis provided by Chan et al. (1997), Saltelli et al. (1999) and Saltelli (2002), sensitivity analysis for autocorrelated variables was defined as the study of how 1) the predicted spatial output for the biological response surface (expected to be equivalent to I_E if responding in the absence of natural variation) differs from the quantified output (I_B) and, 2) how the latter difference and the quantified biological output (I_B) is apportioned between extrinsic mechanisms (I_{be}), intrinsic mechanisms (I_{bi}) and natural variation (I_N) (Table 3.1).

Two complimentary methods of sensitivity analysis were applied here, one graphical and the other statistical (Frey and Patil 2002), to partition the spatial structure in the biological outputs into the amount of variation attributable to these categories of likely mechanisms (Table 3.1). The graphical method (Table 3.1i) involves the use of correlograms and the autocorrelation output, in conjunction with the spatially explicit output quantified using Spatial Analysis by Distance IndicEs (SADIE). The statistical method (Table 3.1ii) uses a combination of Principal Coordinates of Neighbour Matrices regression analysis (Borcard and Legendre 2002, Borcard et al. 2004) and autocorrelation coefficients, from the distance class in which autocorrelation is maximised, to apportion the variability in the biological variables between different categories of mechanisms (Legendre and Legendre 1998, Didier and Porter 2003).

Table 3.1. Two sensitivity analysis methods, their terms, explanations, and derivation, used to quantify the contribution of extrinsic and intrinsic mechanisms and natural variation to determining the total (spatial and other) variability in the biological variable of interest (z).

Method	Term	Explanation	Derivation
i. Graphical[1]: spatial autocorrelation across distance classes			
	I_E	Quantified autocorrelation output, *I*, for the environmental surface	Moran's autocorrelation coefficient, *I*
	I_B	Quantified autocorrelation output, *I*, for the biological response surface (i.e. spatial structure in the biological variable of interest, z)	Moran's autocorrelation coefficient, *I*
	I_{be}	Component of autocorrelation output I_B attributed to extrinsic mechanism	Randomise data to remove effect of I_{bi} on I_B Visualisation using SADIE[2]
	I_{bi}	Component of autocorrelation output I_B attributed to intrinsic mechanisms causing spatial structure in z	$\hat{I}_{bi} = I_B - I_{be}$ Visualisation using SADIE[2]
	I_N	Imperfection in the autocorrelation output of the biological response to spatial structure in the environmental surface, as a consequence of natural variation[3]	Difference between I_E and I_B $1 - (I_B / I_E)$
ii. Statistical[1]: principal coordinates of neighbour matrices (PCNM)[4] and partitioning of autocorrelation (I_B)[5] and spatial components of PCNM			
Step 1 (PCNM)	r^2_S	Variability attributable to spatial structure in z	Proportion of deviance explained by PCNM regression of y on principal coordinates of positive eigenvalues
	r^2_{NSM}	Variability attributable to non-spatially structured mechanisms, if all spatial structure removed by PCNM	$1 - r^2_S$
Step 2 (Partitioning I_B)	I^2_B	Variability expressed by maximised autocorrelation output (*I* value), attributable to extrinsic (here, treatment) and intrinsic mechanisms (here, across fruit oviposition patterns)	$(I_B)^2$
	I^2_{be}	Variability expressed by autocorrelation output, attributable to extrinsic mechanisms alone	$(I_{be})^2$
	I^2_{bi}	Variability expressed by autocorrelation output, attributable to intrinsic mechanisms (in this case across fruit oviposition patterns)	$(I_B)^2 - (I_{be})^2$
Step 3 (Partitioning r^2_S)	$\hat{r}^2_{bo}$	Variability attributable to other spatially structured mechanisms not quantified with spatial autocorrelation in distance class 1[6] (here, oviposition within fruit and structure across distance classes beyond distance class 1)	$r^2_S - I^2_B$
	I_N	Proportion of autocorrelation output in class 1 not realised in z and attributed to natural variation[3]	$1 - (I_B/I_E)$

[1] *sensu* Frey and Patil (2002)
[2] the quantified spatial explicit output of SADIE, Perry et al. (1999); results in Chapter 2
[3] *sensu* Elith et al. (2002)
[4] Borcard and Legendre (2002, 2004)
[5] applied here to results of distance class 1 of autocorrelation analysis only, modification of method used by Didier and Porter (2003)
[6] or class with highest *I* value

Two levels of spatial structuring in the fly assemblage was expected, i.e. a response to the microclimatic heterogeneity induced by the mesocosm (sun and shade plots, extrinsic treatment mechanism), and spatial structuring within individual (aggregation) and across adjacent fruit (likely to result from fly oviposition behaviour) (intrinsic mechanisms) (see Chapter 2, Warren et al. 2003).

In this study, the extrinsic mechanism (I_{be}, the influence of the microclimatic treatment) is equivalent to Legendre et al.'s (2002) 'environmental control model', where the biological response is spatially structured because the explanatory variable is spatially structured. Here, this is the response of the flies to the imposed microclimatic treatment. The imposed microclimatic treatments have been shown to affect the abundance and species richness of this drosophilid assemblage in the predicted manner; namely, the extrinsic mechanisms of the response of flies to the imposed treatment resulting from higher mortality of the flies, and lower oviposition rates, in sun than in shade plots, and therefore lower abundance and species richness in the sun plots has previously been confirmed (Chapter 2).

The category of intrinsic mechanisms generating autocorrelation in the biological output (I_{bi}) include aggregated egg laying in individual fruit and female oviposition in neighbouring fruit, i.e. the component of the spatial output resulting from within and across fruit oviposition (after initial oviposition in a fruit, females are more likely to oviposit in neighbouring fruit) (Heard and Remer 1997, Remer and Heard 1998, Warren et al. 2003). Spatial aggregation across and within fruit has also previously been shown to occur in this assemblage (Chapter 2).

Consequently, the spatial structure that is present in the biological output may be ascribed to a combination of autocorrelation in the response of flies to the extrinsic treatment (I_{be}) and to intrinsic mechanisms (I_{bi}, e.g. oviposition behaviour). To estimate I_{be}, the spatial structure in I_B as a consequence of I_{bi} was removed (Table 3.1). Randomising the values of each biological variable, within all sun and all shade plots, removed across fruit oviposition patterns and maintained the treatment effect, thereby allowing I_{be} to be estimated. The graphical difference between the autocorrelation output of the environmental surface (I_E) and the autocorrelation output of the biological response surface (I_B) represents autocorrelation that is 'not realised' as a consequence of natural variation in the biological responses (I_N) (Table 3.1). The outputs of the biological variables were therefore expected to be weaker than the environmental surface because of I_N (see also Chapter 2).

Graphical sensitivity analysis

The spatial structure (autocorrelation) of the environmental surface was quantified using the rectangular structure of the experiment (the spatial position of all 216 fruit). Each fruit was coded (0,1) as occurring in either a sun (1) or shade (0) plot in the experiment (Fig. 3.1), and these data were used to depict the spatial structure of the environmental surface. The abundance and species richness data for each fruit was pooled across the eleven sampling occasions, to examine the quantified spatial pattern for the duration over which the fruit resource was available. Pooling spatial samples that have been taken over many sampling occasions strengthens the detected spatial structure but the form of the spatial pattern is similar for single and pooled sampling occasions for this mesocosm data (i.e. spatiotemporal mechanism of natural variation does not affect hypothesis generation, Ives and Klopfer 1997, Chapter 2). Also, calculating species abundances over numerous sampling occasions, and deriving occupancy from these abundances, is standard procedure at larger sampling scales (see, for example, Brown et al. 1995, Harrison et al. 1997). Therefore, a total abundance value (all species pooled) and abundance values for each species separately over the entire sampling period were obtained for each fruit. These values were then used to quantify and compare patterns in the biological response surfaces of the assemblage variables (species richness, total abundance) and individual species abundances across the study arena (i.e. the biological variables). Because all emerging flies were counted, the quantified structures are those resulting from the true abundances of the species constituting the assemblage with no sampling effect.

Omnidirectional correlograms (Moran's *I*) were used to examine the spatial structure and to test for significance in autocorrelation coefficients across the study arena for the environmental surface and biological variables (SAAP-PC Version 4.3, Exeter Software, Wartenberg 1989, Legendre and Legendre 1998). Although Radeloff et al. (2000) recommend using directional correlograms when dealing with grid and checkerboard patterns, no anisotropy (directional pattern) in abundances and species richness across the study arena was apparent from initial raw data plots of the biological response surfaces from this study (Chapter 2). Omnidirectional correlograms have also been shown to be sufficient to detect the predicted spatial pattern for this mesocosm assemblage although the omnidirectional output is slightly weaker than the direction in which *I* is maximised (Chapter 2).

In square sampling areas, the x-intercept (where the correlogram becomes negative or zero) approximates the length of one side of the square (Sokal and Wartenberg 1983) and should therefore approximate six fruit neighbours for this experimental design. The biological variables were thus expected to be positively autocorrelated for distance classes 1--4 and 9--13 and negatively autocorrelated for classes >4--<9, corresponding to the sun and shade plot sizes in the experiment, although some resolution of this pattern is lost when using omnidirectional correlograms (Radeloff et al. 2000, Chapter 2). The first four distance classes correspond to the size (width) of a microclimate treatment plot (six fruit neighbours). Distance classes >4--<9 correspond to the distance between adjacent plots (different treatments) (7--12 fruit neighbours), while the distance classes 9--13 correspond to the distances between plots of the same treatment (13--18 fruit neighbours).

Omnidirectional correlograms were constructed using 15 distance classes (obtained using Sturge's rule, Legendre and Legendre 1998) and equal distance intervals (each class therefore represented 0.27 m; equivalent to approximately two adjacent fruit), and the number of point pairs in each distance class thus varied. It is recommended that only half the total distance of the correlogram should be interpreted (Rossi et al. 1992). However, the correlograms fulfilled the minimum requirements for interpretation (point pairs above 1 % of the total number of point pairs) up to distance class ten and have therefore been interpreted for 2/3 of the total distance (Legendre and Fortin 1989, Rossi et al. 1992). Individual autocorrelation statistics are significant when tested against the null hypothesis that I does not differ from its expected value which is $-1/(n-1)$, where n is the number of fruit, according to the test procedure of Cliff and Ord (1981) (Legendre and Legendre 1998). Overall correlogram significance was tested using Bonferroni's correction for multiple comparisons (Rice 1989, Legendre and Legendre 1998).

All abundance and species richness data were transformed ($\log_e (x+1)$) prior to analysis and correlogram construction (to stabilise the variance in the data), whereas environmental surface data (codes (0, 1) of fruit occurring in the sun or shade treatments) was not transformed (Dutilleul and Legendre 1993, Legendre and Legendre 1998). Only species occupying >30 fruit over the sampling period were used in autocorrelation analyses because this is an approximate minimum occurrence necessary (Sokal and Oden 1978, Legendre and Fortin 1989). Therefore, the abundances of *D. melanogaster, D. buzzatii, D. busckii* and *Zaprionus* msg 2 were not considered individually. Correlograms were thus constructed for species richness, *D. simulans*

and *Zaprionus* msg 1, and for the abundances of all species combined. The correlograms represent the quantified autocorrelation outputs of the environmental surface (experimental design) (Fig. 3.1) and biological responses surfaces (Fig. 2.2 in Chapter 2) and are referred to as I_E (environmental) and I_B (biological) output values respectively. Periodicity in a variable is defined here as repeated alternation across the correlogram of significantly positive or significantly negative I values.

The difference in output strength between the environmental and biological outputs was expressed as a proportion of the environmental output and I_N was quantified across distance classes as 1- (I_B/I_E) (Table 3.1). In the graphical sensitivity analysis method (*sensu* Frey and Patil 2002) combinations of the I_E, I_B and I_{be} correlograms are represented to provide a visual indication of I_N and I_{bi} across the ten distance classes.

Because multiple spatial analytical procedures are required to generate realistic hypotheses (Dale et al. 2002, Perry et al. 2002), spatially explicit analyses were performed using Spatial Analysis by Distance IndicEs (SADIE) (Perry et al. 1996, 2002, Perry 1998). SADIE related the biological response at an individual locality to the environmental surface at the same locality (Dale et al. 2002, Perry et al. 1996, 2002, Perry 1998). SADIE was used to determine the degree of aggregation in abundance (total and individual species) across the study arena, as well as to examine spatial aggregation at individual localities (fruit) (Perry 1995, 1998, Perry et al. 1996, 1999). This graphical output was used to confirm the spatial patterns within and across fruit as well as the patterns generated by the extrinsic treatment mechanism. These results are reported in Fig. 2.5 and Table 2.2 (Chapter 2) and have not been included here.

Statistical sensitivity analysis: assessing the relative importance of mechanisms

The statistical sensitivity analysis method (Table 3.1ii) requires three steps to partition spatial variation into likely mechanisms. In all cases 'r^2' is used to represent the proportion of deviance explained by the spatial terms in the Principal Coordinates of Neighbour Matrices (PCNM) model calculated from generalised linear, not ordinary least squares, regression. The square of Moran's I (I^2) represents the proportion of variation explained (by either the extrinsic and/or intrinsic mechanisms) for paired points at a specific distance (Didier and Porter 2003, see below). Although the proportion of explained deviance (r^2, conceptually equivalent to the least squares coefficient of determination, R^2) and I^2 cannot be used to determine cause-and-effect

per se, but rather the proportion of variance (or variation with respect to PCNM regression analysis, Legendre and Legendre 1998) of one variable that is predictable from another variable, knowledge of this system is such that in this case estimated r^2 and I^2 values can be attributed to specific mechanisms. Note, however, that the proportion of variability assigned to different mechanisms is only an estimate that may be used to generate more specific hypotheses pertaining to the importance of individual categories of mechanisms.

The first step in this sensitivity analysis method assesses the contribution of total spatial structure to explaining the variability in each biological variable using Principal Coordinates of Neighbour Matrices regression analysis (PCNM) (Borcard and Legendre 2002) (Table 3.1ii). PCNM has an advantage over trend surface analysis (TS) in that where TS models broad scale spatial structures, PCNM is able to adequately resolve spatial structures over a wide range of scales including fine scale structure (Borcard and Legendre 2002). PCNM analysis therefore presents a more optimal solution to estimating the total spatial structure present in the biological variables for this mesocosm where, not only broad scale structuring is likely to be present, but also finer scale spatial structure is predicted *a priori* (Chapter 2). The PCNM methodology is similar to TS analysis except that positive eigenvalues are used as spatial descriptors instead of a polynomial function of X and Y coordinates (Borcard and Legendre 2002, Borcard et al. 2004). A matrix of Euclidean distances, truncated at four times a specified threshold value (here selected to equal the distance between neighbouring fruit; 0.2 m), is constructed. Next, the principal coordinates of the truncated matrix are computed. The principal coordinates derived from the positive eigenvalues are then used as explanatory variables in, for example, a multiple regression (Borcard and Legendre 2002). PCNM coordinates were calculated using SpaceMaker2[1] (Borcard and Legendre 2002, 2004). Following Borcard and Legendre (2002), these coordinates were then used in a multiple regression to obtain the deviance value accounted for by spatial structure in the biological variable (r^2s) (Table 3.1ii). Generalised linear models were used and a Poisson error structure and log link function were specified (McCullagh and Nelder 1989). The proportion of explained deviance was calculated for each model (for simplicity referred to as r^2 values) (McCullagh and Nelder 1989). If, after PCNM analysis, no spatial structure remains in the residuals (confirmed by compiling correlograms of the

[1] <http://www.fas.umontreal.ca/biol/legendre/>

residuals), the remaining proportion of variation may be attributed to non-spatially structured mechanisms (r^2_{NSM}) (Table 3.1ii).

Following PCNM analysis, I_B was partitioned by squaring Moran's I values (Step 2, Table 3.1ii). Moran's I values are similar to correlation coefficients (Sokal and Oden 1978, Legendre and Legendre 1998), and the square of individual I values represents an estimate of the proportion of variation at a specific distance explained solely by the distance between paired points (Didier and Porter 2003). I values from the distance class in which I is largest (here, distance class 1) were used because this is the distance over which autocorrelation is maximised and likely to contribute the most to explaining spatial pattern in the biological variable. Thus, $(I_B)^2$ values revealed the proportion of variation that was detected by autocorrelation analysis in distance class 1 (I^2_B) (Table 3.1ii, Didier and Porter 2003). This autocorrelation in the output of the biological response variable may be attributable to both I_{be} and I_{bi} for this system (see above explanation, Table 3.1). Squaring I_{be} (value obtained using graphical method) estimated the proportion of variation in the biological variable attributable to the treatment mechanism (I^2_{be}). Because I_{bi} is a derived autocorrelation value, instead of squaring the $\hat{I}_{bi}$ values, the proportion of variation in the biological variable attributable to intrinsic mechanisms was obtained by subtraction (in this case oviposition across fruit, I^2_{bi}) (Table 3.1ii).

The third and final step to the statistical partitioning of variation involves calculating that component of spatial structure that is detected by PCNM analysis, but not detected by autocorrelation in the distance class in which I is maximised (Table 3.1ii). PCNM analysis estimates total spatial structure in a variable. By contrast, in spatial autocorrelation analysis, the value of I in distance class one represents the spatial structure attributable only to the distance between paired sampling points over that distance, i.e. excluding all spatial structure explained by greater distance classes (Legendre and Legendre 1998, Didier and Porter 2003). The spatial structure at an individual sample point cannot be directly assessed through autocorrelation analysis because a single sample point will have no paired points with which to compare it to. Therefore, the difference between r^2_S (total spatial structure present calculated with PCNM analysis) and I^2_B (spatial structure over a fixed distance quantified using autocorrelation analysis) represents the proportion of variation in the biological variable attributable to within sample point structure, as well as spatial structure across the remaining distance classes (other (both extrinsic and intrinsic) mechanisms, $\hat{r}^2_{bo}$, Table 3.1ii). Although this component

comprises two contrasting mechanisms (or sets thereof), it nevertheless indicates if the strength of the combined contribution of these mechanisms to explaining spatial pattern is the same as, weaker or stronger than, the distance over which I is maximised. Finally, the proportion by which the biological spatial response was reduced by natural variation (standardised difference between the biological spatial response and the environmental surface) was then calculated (I_N, Table 3.1).

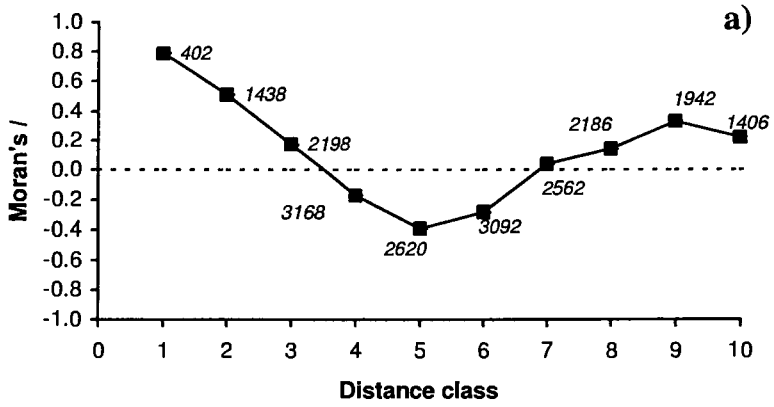
Results

Graphical sensitivity analysis

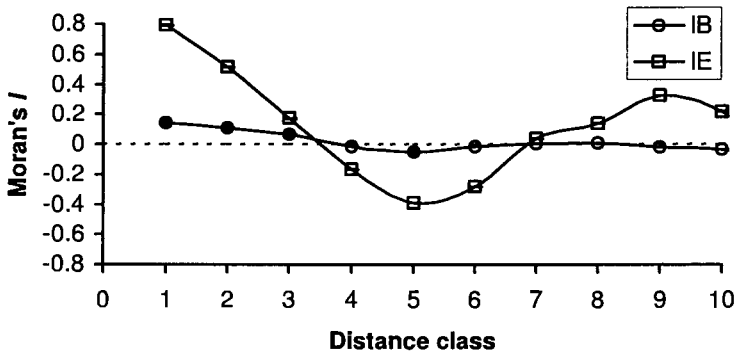
The omnidirectional correlogram of the environmental surface was significantly positively autocorrelated in the first three distance classes (approximately 5 fruit neighbours) and classes 7--10 (approximately 10--14 fruit neighbours), and significantly negatively autocorrelated in classes 4--6 (approximately 6--9 fruit neighbours) (Bonferroni $\alpha = 0.001$; Fig. 3.2a). The x-intercept lay between distance classes 3--4 (or 5--6 fruit neighbours), approximating the length of one side of the square treatment plots. Thus the periodicity present in the omnidirectional autocorrelation profile of the environmental surface reflected plot structure across the experimental arena (Fig. 3.2a, see also directional correlograms in Chapter 2).

The x-intercepts of the correlograms for species richness, abundance, and *D. simulans* and *Zaprionus* msg 1 abundance approximated the x-intercept in the environmental surface (at or between distance classes 3--4, equivalent to 5--6 fruit) (Fig. 3.2a-d, I_B plots). The periodicity apparent in the environmental output was also present in total, *D. simulans* and *Zaprionus* msg 1 abundance, but not in species richness (Fig. 3.2). Distance 5 corresponds to the distance between adjacent sun and shade plots and all the biological variables were found to be less similar than expected by chance (negatively autocorrelated) over this distance (Fig. 3.2b-e). Therefore, the periodicity of the biological output was qualitatively similar to the environmental output, reflecting microclimate plot structure (SADIE results also confirm this, see Fig. 2.5, Chapter 2). The SADIE output also confirmed within and across fruit aggregation patterns probably caused by within and across fruit oviposition (see Fig. 2.5, Chapter 2).

a)


Moran's I
Distance class
402
1438
2198
3168
2620
3092
2562
2186
1942
1406

b)


Moran's I
Distance class
IB
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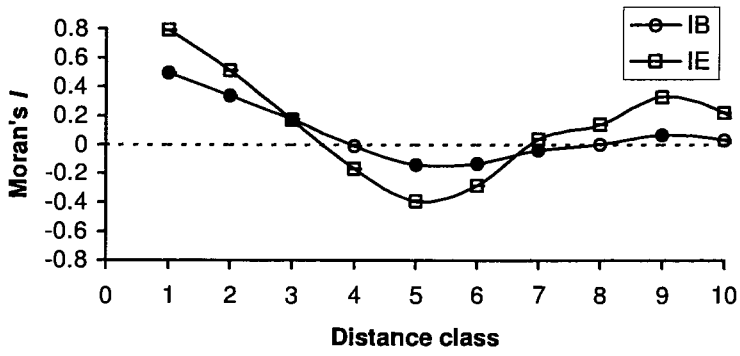
c)


Moran's I
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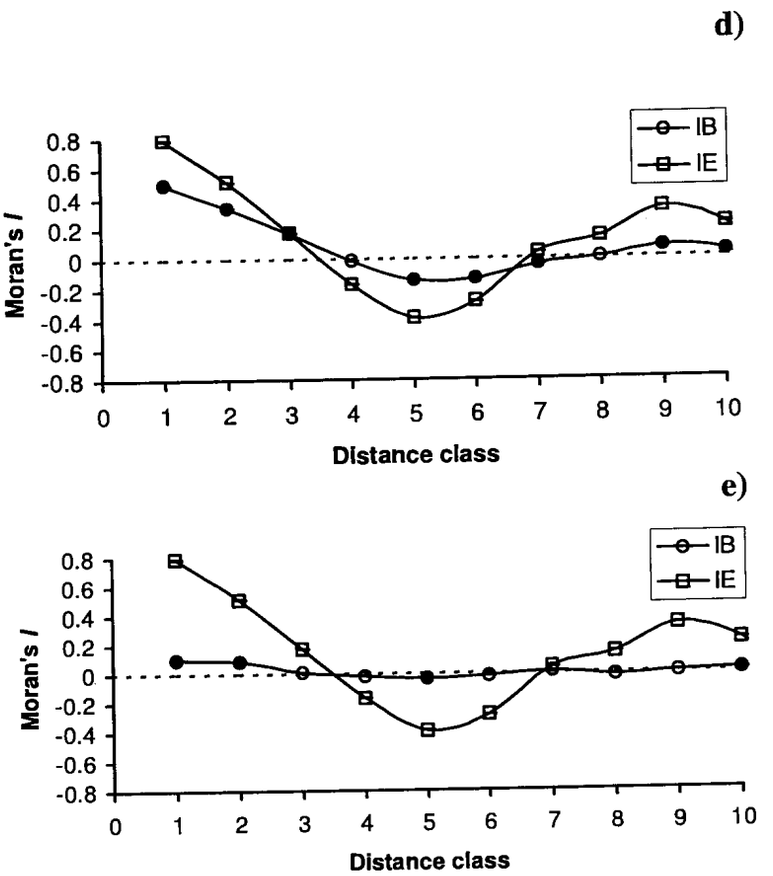

Fig. 3.2. Autocorrelation output (omnidirectional correlograms of Moran's *I*) for a) the environmental surface as determined by the experimental design (I_E), b) species richness, c) total abundance, d) *D. simulans* abundance and e) *Zaprionus* morphospecies group 1 abundance (I_B in each case). Closed points represent significant Moran's *I* values at $P < 0.05$. The number of point pairs in each distance class appears in italics for a) the environmental surface, and are the same throughout. Bonferroni corrected overall correlogram significance levels as follows: a-d: $\alpha = 0.001$ and e: $\alpha = 0.01$. For a-e, error bars are +/- one S.E. of the individual points (too small to be visible in most cases). The difference in *I* between I_E and I_B (realised autocorrelation output for biological response surface attributable to extrinsic and intrinsic mechanisms) is I_N (autocorrelation output for biological variable that was not realised and was attributable to natural variation) (see text for details).

In this study natural variation dampened the biological autocorrelation output by 1-99 % depending on the distance class and variable in question (Fig. 3.2b-e, Fig. 3.3a,b). For example, natural variation dampened species richness *I* values by 62 to 90 %, while total abundance *I* values were dampened between 33 to 99 % for the first 10 distance classes (Fig. 3.3a).

The quantified biological outputs (I_B) were always greater for the first four distance classes than the output attributable to the treatment mechanism alone (I_{be}), except for *Zaprionus* msg 1 where the output was greater for only the first two distance classes (Fig. 3.4a-d). Therefore, although the treatment mechanism (I_{be}) was responsible for spatial structuring in the output of the biological variables, the intrinsic response of across fruit oviposition by the flies (I_{bi}) also contributed to the final quantified spatial output of the biological variables, increasing the strength of the autocorrelation coefficients (Fig. 3.4a-d).

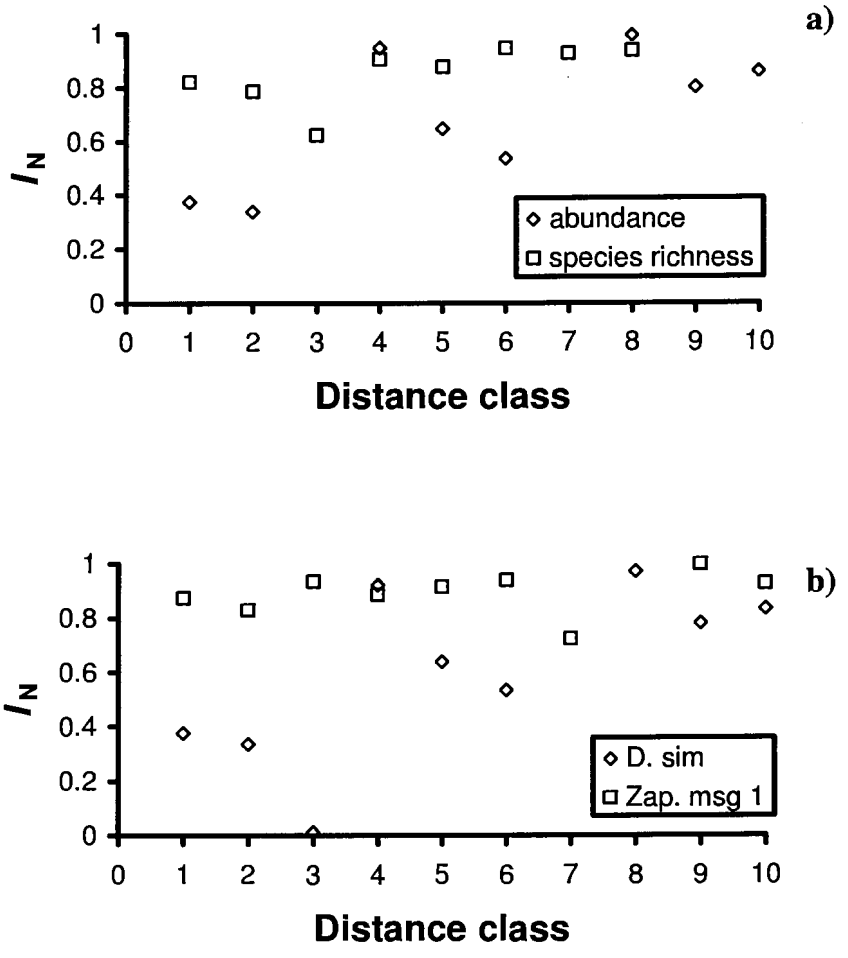

Fig. 3.3. Strength of dampening by natural variation (I_N) on a) assemblage variables and b) individual species abundances (D. sim = *D. simulans* and Zap. msg 1 = *Zaprionus* msg 1). Proportions that tend towards positive 1.0 represent distance classes where the strength of the dampening by I_N on the biological output Moran's I is maximised; missing values for distance classes represent classes where the biological and environmental I values were in the opposite direction (viz. positive and negative autocorrelation) and it would therefore be nonsensical to interpret the size of the output. $I_N = 1 - (I_B/I_E)$.

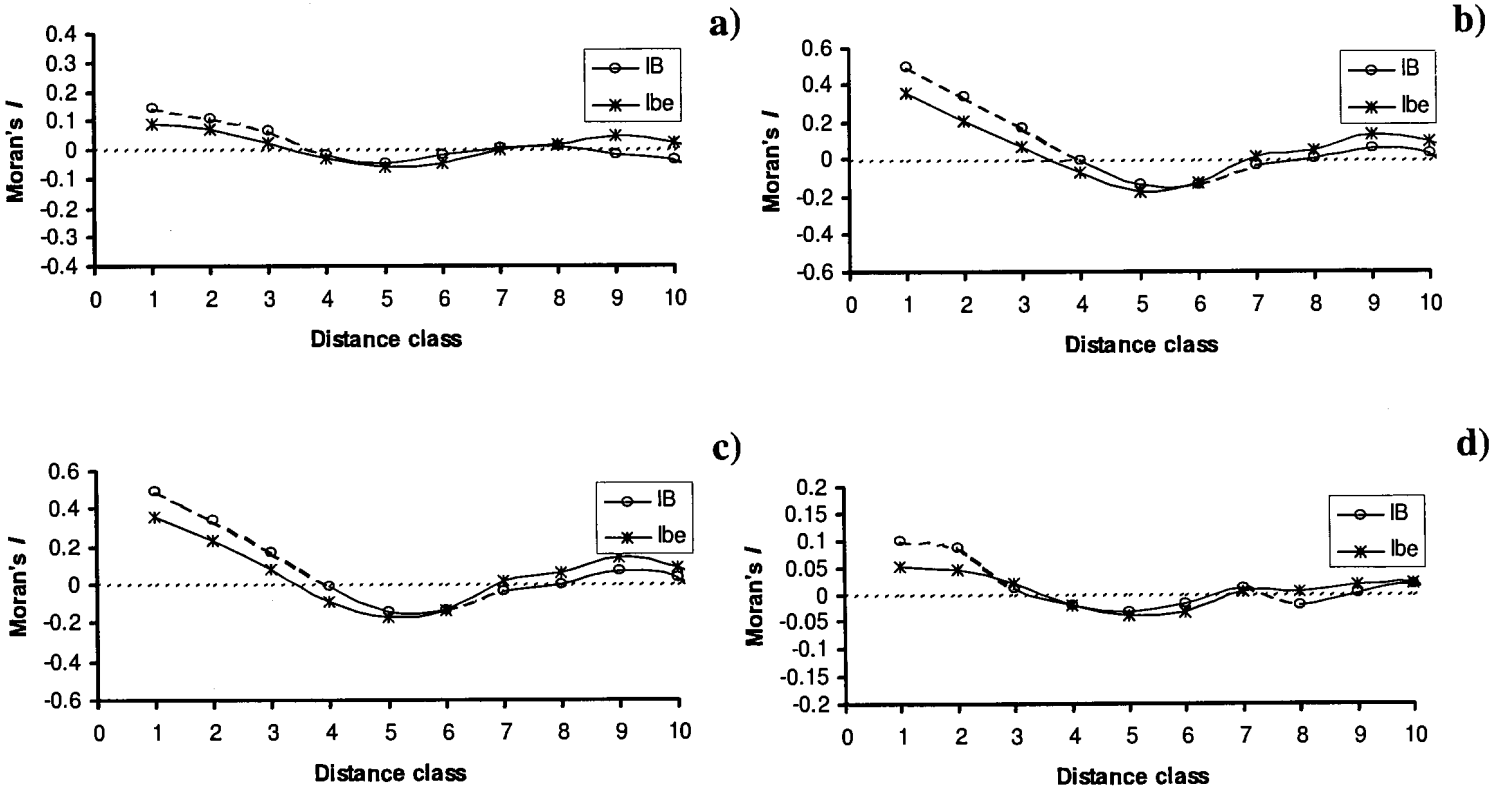

Fig. 3.4. Autocorrelation output (omnidirectional correlograms of Moran's *I*) for a) species richness, b) total abundance, c) *D. simulans* abundance and d) *Zaprionus* morphospecies group 1 abundance. I_B is the observed biological output, I_{be} is the observed output due to the extrinsic (treatment) mechanism alone. The difference in I_B and I_{be} represents the intrinsic mechanism attributable to oviposition across fruit (I_{bi}) (see text for details). Dashed lines represent distances over which the observed biological output is stronger (i.e. more positive or more negative) than the output attributable to the treatment mechanism alone.

Statistical sensitivity analysis

Natural variation caused a 38-87 % reduction in the autocorrelation outputs of the biological variables in the first distance class (where I_B was maximised) compared with the environmental output (Table 3.2). The reduction in the autocorrelation response of total and *D. simulans* abundance was lower than in other biological variables (Table 3.2). In all cases, natural variation caused a > 30 % weakening in the biological outputs (Table 3.2).

The PCNM regressions revealed that 14-44 % of the variation in the biological variables was attributable to spatial structure, while the remaining 56-86 % of the variation was attributable to non-spatially structured mechanisms (Table 3.2, r^2_S, r^2_{NSM}). Most of the spatial structure present in total and *D. simulans* abundance was present in distance class 1 of the outputs, i.e. the dominant spatial mechanism (or mechanisms) was acting across adjacent fruit (Table 3.2, I^2_B). By contrast, most of the spatial structure for species richness and *Zaprionus* msg 1 was explained by other mechanisms, namely within fruit structure and structuring at greater distance classes (Table 3.2, $\hat{r}^2_{bo}$). The treatment mechanism contributed to explaining variation in the autocorrelation of the biological outputs (Table 3.2, I^2_{be}). This mechanism (mortality effect and lower oviposition rates in sun treatment) explained 13 % of the quantified output for total and *D. simulans* abundance (Table 3.2). The proportion of the quantified output attributable to the treatment mechanism was only 1 % for species richness, and less than 1 % for *Zaprionus* msg 1 (Table 3.2). The difference between I_B and I_{be} may be attributed to oviposition across fruit (I_{bi}) and the proportion of autocorrelation explained by this mechanism was higher (I^2_{bi} = 0.11) for total and *D. simulans* abundance than species richness and *Zaprionus* msg 1 (Table 3.2). Except for *Zaprionus* msg 1, oviposition patterns across fruit explained the same or less of the variation in the spatial output of the biological variables than the treatment mechanism (Table 3.2). Therefore, although the treatment and oviposition across fruit mechanisms contributed the most to explaining total spatial structure of total abundance and *D. simulans* abundance, for species richness and *Zaprionus* msg 1 abundance the component of spatial structure not detected by the first distance class ($\hat{r}^2_{bo}$) contributed the most to explaining the observed spatial structure (Table 3.2).

Although the correlograms of the residuals of *D. simulans* and *Zaprionus* msg 1 after PCNM were significantly autocorrelated, only one *I* value on each correlogram was significant at the Bonferroni corrected level ($\alpha < 0.003$) (Table 3.2). In addition, residual correlograms appeared similar to Legendre and Fortin's (1989) 'random number' correlogram (results not shown). Therefore, r^2_{NSM} for *D. simulans* and *Zaprionus* msg 1 represented mainly non-spatially

structured multiple mechanisms, as well as a very small spatially structured component. Consequently, a minor component of the multiple mechanisms causing variability in the biological variables remained spatially structured after PCNM, although this spatial structure was significantly weaker than before PCNM regression.

Table 3.2. Results of statistical sensitivity analysis to apportion variation in the biological output to categories of likely mechanisms. Proportion reduction in biological autocorrelation attributed to natural variation (I_N). Estimates of the proportion of variation in the biological variables explained by non-spatially structured mechanisms (r^2_{NSM}) and the total variation explained by spatial structuring of the biological variables (r^2_S) apportioned between the variation that is expressed by the autocorrelation output of the variables (I^2_B), extrinsic (I^2_{be}), intrinsic (I^2_{bi}) and other ($\hat{r}^2_{bo}$) categories of mechanisms. r^2 (proportion of explained deviance for the PCNM model) and I^2 (proportion of variability expressed by autocorrelation output and attributable to particular mechanism) (see text for details). $r^2_{NSM} + r^2_S = 1.0$. No significance levels are given because these will be falsely inflated by autocorrelation (Legendre and Legendre 1998).

Variable	I_N	r^2_{NSM}	r^2_S	I^2_B	I^2_{be}	I^2_{bi}	$\hat{r}^2_{bo}$
Abundance	0.38	0.56[1]	0.44	0.24	0.13	0.11	0.20
Species richness	0.82	0.86[1]	0.14	0.02	0.01	0.01	0.12
D. simulans	0.38	0.57[2]	0.43	0.24	0.13	0.11	0.19
Zaprionus msg 1	0.87	0.84[2]	0.16	0.01	< 0.01	0.01	0.15

[1] no spatial structure remains in the residuals, thus this component represents truly non-spatial mechanistic structure

[2] significant autocorrelation in residuals at $P < 0.05$ for one point on each correlogram: class 10 and 3 respectively; residual autocorrelation plots appear random

Discussion

The sensitivity analysis method developed here improved the hypothesis generating capacity of spatial analysis. Natural variation in the multiple mechanisms structuring the biological responses was identified as weakening the spatial outputs by 38-87 % (although approximately 5 % of this variation resulted from the use of omnidirectional, instead of, directional correlograms (Chapter 2)). The application of the graphical sensitivity analysis confirmed spatial structure in the biotic variables resulting from the extrinsic treatment mechanism and intrinsic within and across fruit oviposition mechanisms. In addition, distinguishing between different categories of identified mechanisms allowed the relative contribution of each mechanism to the observed pattern to be assessed. For example, just over half of the spatial structure present in total and *D. simulans* abundance was explained by intrinsic across fruit oviposition. The dominant spatial mechanism(s) structuring species richness and *Zaprionus* msg 1 was acting within individual fruit and over distances greater than the distance between neighbouring fruit.

Because the intrinsic and extrinsic mechanisms were separated from each other in the sensitivity analysis, it was possible to demonstrate that the strength of spatial structure was inflated by intrinsic mechanisms. Abiotic (extrinsic) mechanisms are known to generate strong spatial structure in biological variables (e.g. Brewer and Gaston 2002). However, intrinsic responses of species may also generate spatial structure that may initially be hidden by strong extrinsic mechanisms and may therefore be thought to play a minor role in generating the observed spatial pattern (see McGeoch and Price 2004). Intrinsic mechanisms are likely to act at finer spatial scales, such as the maximum dispersal distance of a species, or even finer movement patterns reflecting an individual's behaviour, than extrinsic mechanisms such as climate. For example, Bowman et al. (2000) found that the spatial variability in small mammal abundance occurred at spatial scales (133-533 m) relevant to the species' dispersal distances. For drosophilids, such fine spatial scales include the movements of flies over distances across individual fruit and trees (e.g. Heard and Remer 1997, Remer and Heard 1998, Inouye 1999), although the individuals of some species disperse over larger distances (Coyne et al. 1982).

A technical issue pertaining to the use of the sensitivity analysis approach outlined here is apparent. PCNM regression analysis was unable to remove all spatial structure present in *D. simulans* and *Zaprionus* msg 1 abundance. This, however, is unlikely to hinder the usefulness of the sensitivity analysis method developed here. First, PCNM analysis is able to detect spatial structure at a wide range of spatial scales, particularly at scales less than the extent of the study (Borcard and Legendre 2002, e.g. PCNM removes more spatial structure than TS for the

mesocosm data used in this study, results not shown). Second, significant spatial structure resulted from a single, significant autocorrelation coefficient on each correlogram. Third, the residual plots appeared random (see Legendre and Fortin 1989). Finally, as with any such methods, one of two approaches to reducing the number of spatial variables included in the final model may be applied. On the one hand, the maximum number of spatial variables may be included in the model (all spatial structure should then be accounted for) (Borcard and Legendre 2002). This leads to an inflated R^2 value by chance alone because of the large number of terms included in the model (Borcard and Legendre 2002). On the other hand, a more parsimonious approach is to reduce the number of terms included in the model by, for example, a stepwise procedure (Borcard and Legendre 2002). The parsimonious approach may then exclude a spatial term that contributes almost negligibly to spatial structure. This is likely to be the case for *D. simulans* and *Zaprionus* msg 1 where spatial structure in the correlogram resulted from a single significant autocorrelation value. Therefore, although PCNM was unable to remove all spatial structure in two of the biological variables, this did not weaken the usefulness of the sensitivity analysis method to identify mechanisms and partition variation.

Wiens (2000), Dale et al. (2002) and Perry et al. (2002) suggest using a range of spatial analytical procedures to generate realistic hypotheses. However, it is possible to combine this proposal with variation partitioning and thereby improve the capacity of spatial methods to generate hypotheses. The sensitivity analysis approach applied here does just that. Multiple graphical methods generated similar hypotheses, supporting the presence of specific mechanisms. Additional hypotheses were generated through the use of more than a single spatial method. And, finally, partitioning the strength of spatial structure into different components of likely spatial mechanisms was possible using the sensitivity analysis approach. The sensitivity analysis approach developed here is therefore advocated as a means to improve understanding of empirical spatial pattern through an enhanced hypothesis generating capacity.

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CHAPTER 4

Predicting abundance from occupancy: a test for an aggregated insect assemblage[1]

Introduction

The ubiquity of positive abundance-occupancy relationships has engendered considerable interest in the causes of this widespread relationship. Indeed, both theoretical and empirical explanations for the relationship abound (see Gaston et al. 1997, Holt et al. 2002a for review). Ecological mechanisms include differences in resource availability, species resource breadth, population characteristics and biotic interactions (e.g. Brown 1984, Warren and Gaston 1997, Gaston et al. 2000, Holt et al. 2002b), while several mechanisms based on the spatial distribution of individuals have also been proposed (Hanski et al. 1993, He and Gaston 2000a,b, Holt et al. 2002a). The relationship also has considerable intrinsic value because it can be used to predict species abundance levels from measures of occupancy (Kunin 1998, He and Gaston 2000a). Successful prediction of this kind has important potential application in conservation assessment and monitoring (Kunin 1998, He and Gaston 2000a,b), and in the prediction of insect pest population densities from incidence measures in agroecosystems (Legg et al. 1992, Peng and Brewer 1994, Perry 1995).

To predict species abundance from occupancy an appropriate description of the underlying spatial distribution of its individuals is required. The negative binomial distribution (NBD) has most frequently been used, because individuals commonly have contagious distributions (Pielou 1977, Taylor et al. 1978, Legg et al. 1992). However, the relationship between abundance and occupancy is also well described by a range of other models, including poisson, power and logistic models, as well as variations thereof (Bliss and Fisher 1953, Pielou 1977, Nachman 1981, He et al. 2002, Holt et al. 2002a). Recently, Kunin (1998) and Harte and colleagues (Harte et al. 1999, 2001) suggested that self-similarity, and the comparison of abundance–occupancy patterns across spatial scales, might be used to further understand the relationship. Taking Kunin's (1998) rationale of using cross-scale occupancy comparisons a step further, He and Gaston (2000a) derived a model (hereafter the HG model) from the NBD to predict the abundances of species from measures of their occupancy. He and Gaston (2000a) parameterise the model using two spatial scales of occupancy data to estimate species

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abundance, simultaneously estimating the parameter k for the sampling extent of interest (hereafter the HG (parameterisation) method). As the degree of aggregation of individuals increases, the parameter k of the NBD declines. Because k is estimated as part of the parameterisation process, the model is generalisable to situations across the continuum of regular, random to aggregated species distributions (He and Gaston 2000a). In contrast to all previous models, the HG parameterisation method requires no *a priori* estimates of aggregation, slope or intercept parameters for the abundance-occupancy relationship. The model is thus considered to be appropriate across a wide range of biological situations (He and Gaston 2002a,b). Indeed, the HG model was used to successfully predict abundance levels from occupancy for tree species (at a local scale) and passerine birds (at a regional scale) (He and Gaston 2000a,b, Kunin et al. 2000).

Nonetheless, He and Gaston (2000b) have pointed out that the model has several limitations. For example, the efficiency of the model declines with increasing grain and extent, and model predictions are therefore expected to be best at fine scales. The value of the aggregation parameter, k, estimated by the HG method is thus sensitive to sampling extent (He and Gaston 2000b). The value tends to increase with spatial scale and this results in a simultaneous, disproportionate increase in the degree to which abundance is underestimated, i.e. at large scales species are predicted to be less aggregated, and abundances therefore lower, by the HG method than they are in reality (Kunin et al. 2000).

In addition, defining the most appropriate 'finest' sampling unit (also called minimum mapping units (MMUs)) for species remains problematic (He and Gaston 2000a, Kunin et al. 2000). The finest possible MMUs for a species would be those that are equivalent to the size of its individuals, where any occupancy measure would provide an exact measure of its abundance. Such an approach is sampling intensive and most often impracticable. In addition, MMUs defined in this way will also not necessarily permit comparisons between species of different sizes from the same assemblage (McGeoch and Gaston 2002). It is therefore not surprising that most species occupancy data are collected at scales far coarser than the size of individuals of the species concerned (McGeoch and Gaston 2002). Coarse-scale MMUs, however, fail to discern fine-scale distribution patterns (e.g. intraspecific aggregation), resulting in either over- or underestimates of abundance (Thomas and Abery 1995, He and Gaston 2000a, Kunin et al. 2000). Therefore, a compromise must be reached between sampling scales that are practical, and those that will provide a satisfactory estimate of species abundance.

The relative efficiency with which the HG method is able to predict abundance is also

likely to differ for rare and common species. In butterflies, declines in the abundance of common species have been shown to go undetected at large scales, while the range sizes of rare species are overestimated at coarse scales (Thomas and Abery 1995, Cowley et al. 1999). Furthermore, k of the negative binomial distribution is known to be sensitive to species density and may need to be adjusted accordingly (Taylor et al. 1979, but see Peng and Brewer 1994). Therefore, the HG method is unlikely to predict the abundance of rare and common species (Gaston 1994a) equally well at a particular scale.

Despite these potential drawbacks, the HG method is the only one available for predicting local species abundances in the absence of information on their aggregation or mean density (the model also appears to fit local scale data better than the fractal model of Kunin (Kunin et al. 2000)). In consequence, it has a wide range of potential applications in macroecology, conservation biology, and pest management (Gaston 1999). However, despite its potential importance, it remains to be empirically tested across a broad range of taxa and spatial scales. Indeed, with the exception of the tests based on the Pasoh tree and Bedfordshire passerine data, few other tests of this model and its parameterisation method have been undertaken (He and Gaston 2000a). The objective here is, therefore, to provide an empirical test of the HG model and parameterisation method using a Drosophilidae assemblage that is an exemplar of many systems characterised by a highly aggregated spatial distribution at local scales (Atkinson and Shorrocks 1984, Rosewell et al. 1990). First, whether the spatial distributions of the species follow the NBD is determined; providing *a priori* justification of the suitability of using the HG model. Then the accuracy of the abundance predictions of the HG model for the species in this assemblage is tested, defining the finest MMU as the single resource unit, i.e. a decaying fruit. Using accurate occupancy records determined in a mesocosm at three spatial scales (MMUs), it is tested whether the abundances of rare and common species in the assemblage are predicted equally well. The effect of substituting k values, calculated independently of the HG parameterisation method, on model predictions are also assessed. Finally, the effect of increasing MMU size on the accuracy of the abundance prediction is evaluated by determining the scale at which the HG model prediction most closely matches the measured abundance of each species.

Materials and Methods

A mesocosm was used to empirically test the abundance-occupancy model. Mesocosms have been found useful in community and macroecology because they are able to generate assemblages similar to those expected under non-experimental, field conditions (Warren and Gaston 1997, Relyea and Yurewicz 2002).

A Drosophilidae assemblage that inhabits decaying fruit (in this case nectarines (*Prunus persicae* Miller variety *nectarina*: Rosaceae)) was used. The fruit were washed and individually weighed before being placed in the field. Variation in fruit mass was small (Mean ± S.E. (g): 58.84 ± 0.05). Three small punctures were made in the skin of each fruit, because Drosophilidae do not lay on unbroken fruit surfaces (Feder and Krebs 1998). Five nectarines were randomly selected for insecticide residue tests and were found to have no detectable levels of residues of chemicals used in the local soft fruit industry (South African Bureau of Standards).

A wire table (2.4 m x 3.6 m; 0.7m high) was placed on the University of Pretoria's Experimental Farm in Pretoria, South Africa (25°45.178"S, 28°15.293"E; Fig. 4.1). The table was placed inside a cage covered with pigeon wire to exclude birds, fruit-piercing moths and large wasps, while allowing the flies ready access to the fruit. Ants were also excluded by the application of grease to the table legs. The table was divided into six equal plots with each plot supporting 36 nectarines (n = 216 nectarines) (Fig. 4.1). Three of the plots in alternate rows of the two columns were artificially shaded with 80 % shade netting to impose a level of microclimatic heterogeneity on the experiment (Fig. 4.1). The nectarines were placed 20 cm apart in a regular grid of 12 x 18 fruit, resembling a regular arrangement of plants in a field, where each plant would be the unit from which presence or absence (occupancy) of a species would be determined. Each fruit was placed on a plastic mesh in the centre of a round plastic container (~ 15 cm diameter and 8 cm deep) containing washed, moist sand. Drosophilidae larvae drop down into the substrate under the fruit when they are ready to pupate after 4.5 to 8.5 days at 25 °C and 80 % RH (Sevenster and Van Alphen 1993). Larvae were therefore allowed to pupate in moist sand underneath the fruit. Starting from the fifth day after exposure of the fruit, the sand containing the fly pupae was removed and placed in 350 ml plastic jars every second day for 25 days in November 1998. The plastic jars were then taken to the laboratory and the emerging flies were identified (according to McEvey et al. 1988). Fresh, moist sand was immediately placed in the containers under the fruit at each collection.

Flies pupated over a period of 19 days (day 7 – day 25). Fly emergence was 2.1 times higher on the 23rd day than any other pupation date and the distribution-abundance patterns of

individuals pupating on this date are examined here. The data used thus represent the largest cohort of flies simultaneously present in the fruit. At least six Drosophilidae species were found. However, specimens belonging to the genus *Zaprionus* could not be identified to species level. The identified species were: *Drosophila simulans* Sturtevant, *D. melanogaster* Meigen, *D. busckii* Coquillett, *D. buzzatii* Patterson and Wheeler, *Zaprionus* morphospecies group 1 (*Zaprionus* msg 1) (which may include both *Z. tuberculatus* Malloch and *Z. sepsoides* Duda) and *Zaprionus* morphospecies group 2 (*Zaprionus* msg 2) (which may include *Z. vittiger* Coquillett and *Z. indianus* Gupta) (McEvey et al. 1988). Damaged specimens (n = 55 of 3029 flies) that could not be identified to species level were removed from subsequent analyses. However, 50 of these specimens were either *D. simulans* or *D. melanogaster*, and are most likely to have been the former because this species was overwhelmingly dominant. The remaining 5 individuals were all *Zaprionus* species. The total measured abundance of each species was calculated by summing the abundance values across all 216 fruit for each of the six species. Species constituting < 5 % of all individuals caught were considered rare. Occupancy (number of MMU's occupied on the table x size of individual unit of a MMU in m^2) was calculated for each species in three MMU sizes: 1X1 fruit (0.04 m^2; n = 216 'grid cells'), 2X2 fruit (0.16 m^2; n = 54 'grid cells') and 3X3 fruit (0.36 m^2; n = 24 'grid cells'). Sampling areas larger than this greatly reduced the number of grid cells (n = 12 for a MMU size of 4X4 fruit) and occupancy was therefore not calculated for sampling areas larger than 0.36 m^2.

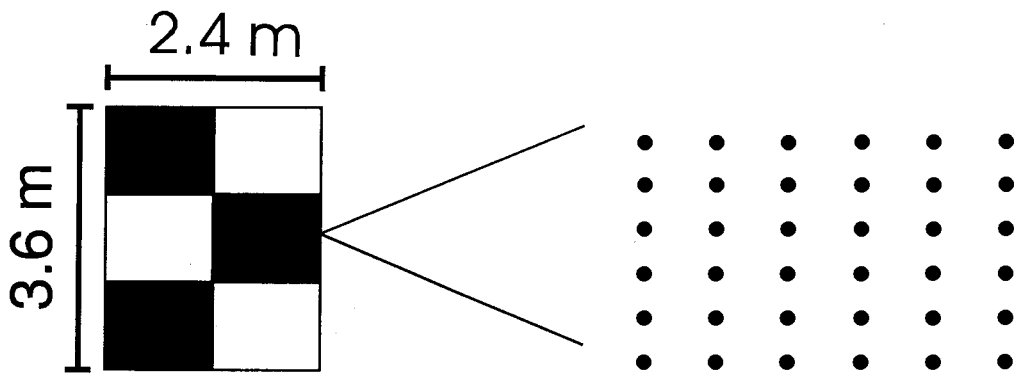

Fig. 4.1. Experimental plot depicting the layout of decaying fruit. Dark blocks represent plots that were shaded with 80 % shade netting. Each plot contained 36 nectarines spaced 20 cm apart in a regular grid as on the right hand side of the figure.

The distributions of the species were tested against the negative binomial distribution (Bliss and Fisher 1953), to determine the suitability of using the HG model to predict the abundances of species in this assemblage. *Drosophila busckii* and *D. buzzatii* were not tested because these species occupied < 1 % of the total number of fruit available for colonisation (n = 216). The significance of the difference between the observed and expected second moments calculated from

$$U = s^2 - (\bar{x} + \bar{x}^2/\hat{k}_2) \qquad \text{eqn 1}$$

is determined by comparison with its standard error (Bliss and Fisher 1953). Values of U falling within the range of the standard error indicate that the distribution of individuals fits the negative binomial distribution.

The HG model was then used to estimate the abundance of each species, across all samples, using the formula

$$\hat{N} = Ak/a\,[(1-A_a/A)^{-1/k}-1] \qquad \text{eqn 2}$$

where A is the extent of the sampling area (for this study 2.4 m x 3.6 m = 8.64 m^2), a is the size of the sampling area or MMU (0.04 m^2, 0.16 m^2, 0.36 m^2 for each scale used) and A_a is the area of occupancy (MMU size x number of occupied MMU) (He and Gaston 2000a). Here, A_a is the measured occupancy (free of sampling error) for each fly species because all flies in each fruit were counted and identified (including those of the rare species). Therefore, complete abundance and occupancy records were used that were not subject to sampling artefacts. Abundance ($\hat{N}$) and k (aggregation parameter) are solved simultaneously in equation 2, using the Newton-Raphson iteration method (Mathews 1987), for each of two MMU's (spatial scales) for each species (the HG method). MMU pairs that were used to estimate abundance and k for each species were i) 0.04 m^2 and 0.16 m^2, ii) 0.04 m^2 and 0.36 m^2, and iii) 0.16 m^2 and 0.36 m^2. To determine if the estimated abundances for each species were similar for the three sets of MMU pairs, they were compared using Sign tests (Sokal and Rohlf 1998). Predicted abundances were compared to real abundances using a Chi-square test (Sokal and Rohlf 1998). The accuracy of the HG method was calculated as predicted abundance/real abundance. Values closest to unity indicate predicted abundances that are equivalent to real abundances and are therefore the most accurate. Values below and above 1.0 represent under- and over-estimation of abundance respectively. Spearman's rank correlation was used to determine if there was a significant relationship between method prediction accuracy and real species abundance (Sokal and Rohlf

1998). The presence of a significant relationship therefore demonstrates that rare and common species are not predicted with equal accuracy by the method in question.

To adjust *k* for species density, *k* was also calculated for each species using the following equation modified from Taylor et al. (1979) (see Shorrocks and Rosewell 1986):

$$1/k = a'm^{(b'-2)} - m^{-1} \qquad \text{eqn 3}$$

where *m* is the mean number of animals per fruit and *a'* and *b'* are constants (*a'* = 8.48; *b'* = 1.51 for drosophilids obtained from Shorrocks and Rosewell 1986) (see also Taylor et al. 1978). These *k* values (assuming a common *k* across scales; He and Gaston 2000a, Kunin 1998) were then substituted into equation 2 to obtain a second estimate of abundance at each of the three MMU's (0.04 m^2, 0.16 m^2 and 0.36 m^2). These abundance estimates were then also compared to the real abundance values using a Chi-square test, and Spearman's rank correlation to determine if there was a significant relationship between prediction accuracy (predicted abundance/measured abundance) and species abundance for each MMU (Sokal and Rohlf 1998).

Finally, a third value of *k* was calculated; using the NBD (k_{NBD}) with a maximum likelihood solution (Bliss and Fisher 1953) for each of the species with > 1 % occupancy. This was then substituted into equation 2 to obtain a third abundance prediction for each species. These abundance estimates were again compared to the real abundance values using a Chi-square test (Sokal and Rohlf 1998).

To distinguish between the three *k* values and abundance estimates obtained the following is used i) k_{HG} and k_{HG} -abundance for those derived using the HG parameterisation method, ii) k_{adj} and k_{adj} -abundance for those obtained by substituting *k* adjusted (Taylor et al. 1979) into the HG model, and iii) k_{NBD} and k_{NBD} -abundance for those obtained by substituting *k* of the NBD (Bliss and Fisher 1953) into the HG model.

Results

None of the species abundance distributions differed significantly from a negative binomial distribution (Table 4.1), and the HG model was thus considered appropriate for estimating the abundances of species in the drosophilid assemblage. Species occupying fewer than 1 % of the fruit available for colonisation, namely *D. buzzatii* and *D. busckii*, were not considered because their occupancies were too low to assess the form of their spatial distribution. Of the six species found, *Drosophila simulans* was the only common species, whereas the remainder were rare (each contributing < 2 % to the total number of sampled individuals) (Table 4.2).

Estimates of abundance and *k* did not converge to a constant root using the HG method

for *D. melanogaster, D.buzzatii* and *D. busckii* for any combination of scales. Although a range of appropriate initial *k* values were used in the iteration process (1; 0.1; 0.01; 0.001; based on previously reported values for drosophilid species, see discussion), the estimated *k* value fluctuated by up to 14 orders of magnitude depending on the initial value of *k* used. The inability of the iteration process to converge to a constant root for these species results from the slope of the derivative of the function being small, and its tangent almost horizontal to the curve (Mathews 1987). One common and two rare species were thus used to test the accuracy of the HG model and parameterisation method.

An abundance estimate could also only be obtained for the smallest combination of MMU's (0.04 m^2 and 0.16 m^2) for *Zaprionus* species group 2 (Table 4.2i). The predicted k_{HG} -abundances obtained for the three MMU pairs did not differ significantly from one another for either *D. simulans* or *Zaprionus* species group 1 (Sign tests for both species: $Z = -0.707$; $P > 0.05$). However, all predicted abundance estimates derived using the HG method differed significantly from the measured abundance values (Table 4.2i). The HG method consistently underestimated (all values are below one) the abundances of species at all MMU combinations (Fig. 4.2a). The greatest disparity occurred for the most abundant species (*D. simulans*), which was underestimated by approximately 94 % for all MMU combinations (Table 4.2i). The accuracy of abundance estimates also declined significantly with an increase in real abundance ($r_s = -0.93$; $t(5) = -5.48$; $P < 0.01$).

Substituting the k_{adj} values into the equation for the HG model yielded abundance estimates for the species that were closer to reality than the k_{HG} -abundance estimates, though they remained significantly different from the measured abundance values for all species (Table 4.2ii, Fig. 4.2b). In all cases k_{adj} values were an order of magnitude smaller than k_{HG} (i.e. the species were more aggregated than the original method predicted) (Table 4.3). As expected, when k_{adj} was substituted into equation 2 the method accuracy was not sensitive to the abundance of the species. That is, the abundances of rare and common species were predicted with equal accuracy after taking the influence of species density on *k* into account (MMU 0.04m^2: $r_s = -0.31$, $t(4) = -0.66$, $P = 0.54$; MMU 0.16m^2: $r_s = 0.26$, $t(4) = 0.53$, $P = 0.62$; MMU 0.36m^2: $r_s = 0.60$, $t(4) = 1.5$, $P = 0.21$). Therefore, the HG method using k_{HG} did not provide accurate predicted abundance values for the species in this assemblage, and the abundances of common species were less accurately predicted (underestimated) than those of the rare species. When using k_{adj} in the model the abundance prediction improved. Although these predictions remained significantly different from measured abundance, rare and common species were predicted with

equal accuracy.

Substituting k_{NBD} into equation 2 yielded highly accurate abundance predictions that were not significantly different from the real abundance values at the finest MMU (Table 4.2iii; Fig 4.2b). Predicted k_{NBD} -abundances for the larger MMU's (0.16 m^2 and 0.36 m^2) were, however, significant overestimates of abundances for all four species (Table 4.2iii).

Table 4.1. The test statistic *U*, the variance of *U* (V (*U*)) and its SE (SE (*U*)) used to determine if species distributions deviated from the negative binomial distribution. The values of *U* fall within the range given by the SE (*U*) and, therefore, the species distributions follow the negative binomial distribution (Bliss and Fisher 1953) (msg= morphospecies group).

Species	U	V (*U*)	SE (*U*)
Drosophila simulans	-150.64	3.98×10^{6}	1996.19
Zaprionus msg 1	-0.46	0.26	0.51
D. melanogaster	-0.02	8.77×10^{-4}	0.03
Zaprionus msg 2	-0.01	9.60×10^{-5}	0.01

Table 4.2. Measured occupancy (number of fruit occupied), abundance (N; % relative abundance) and mean abundance per fruit (± S.D.) for each species in the assemblage (Dsim= *Drosophila simulans*; Zap1 = *Zaprionus* msg 1; Dmel = *D. melanogaster*; Zap2 = *Zaprionus* msg 2; Dbuzz = *D. buzzatii*; Dbusck = *D. busckii*). Predicted abundance using i) the original method of He and Gaston (2000a) to solve for abundance and k_{HG} simultaneously, ii) predicted HG k_{adj} abundance† and iii) predicted HG k_{NBD} abundance‡ for three minimum mapping units (MMU's). Dashes indicate either no convergence at a solution for abundance and k, or abundances were not predicted for these species (see text). Values in parenthesis indicate results of χ^2 analyses for the differences between real and predicted abundances in the following order: df; χ^2 value; significance level. *** = P < 0.001.

Species	Measured Occup.	N (%)	Mean N (±S.D.)	**i. Predicted HGk_{HG} Abundance**		
MMU pair				0.04 m^2; 0.16 m^2	0.04 m^2; 0.36 m^2	0.16 m^2; 0.36 m^2
				(2; 2531.86; ***)	(1; 2542.75; ***)	(1; 2586.52; ***)
Dsim	102	2869 (96.50)	13.28 ± 33.51	184.04	177.87	153.36
Zap1	21	56 (1.8)	0.28 ± 1.06	23.46	23.83	26.01
Dmel	17	24 (0.80)	0.11 ± 0.43	-	-	-
Zap2	8	10 (0.34)	0.05 ± 0.25	8.40	-	-
Dbuzz	1	9 (0.30)	0.04 ± 0.61	-	-	-
Dbusck	2	2 (<0.001)	0.01 ± 0.10	-	-	-
				ii. Predicted HGk_{adj} Abundance†		
MMU				0.04 m^2	0.16 m^2	0.36 m^2
				(5; 2287.52; ***)	(5; 35147.98; ***)	(5; 11665x10^7; ***)
Dsim	102	2869	13.28 ± 33.51	316.06	902.07	3239.60
Zap1	21	56	0.28 ± 1.06	44.99	352.47	4262.51
Dmel	17	24	0.11 ± 0.43	36.42	902.91	1673229.00
Zap2	8	10	0.05 ± 0.25	11.33	29.37	442.73
Dbuzz	1	9	0.04 ± 0.61	1.04	2.81	4.59
Dbusck	2	2	0.01 ± 0.10	1.80	0.81	0.64
				iii. Predicted HGk_{NBD} Abundance‡		
MMU				0.04 m^2	0.16 m^2	0.36 m^2
				(3; 0.38; ns)	(3; 1469x10^5; ***)	(3; 103113x10^8; ***)
Dsim	102	2869	13.28 ± 33.51	2874.27	6.52 x 10^5	1.72x10^8
Zap1	21	56	0.28 ± 1.06	60.54	1336.64	54256.75
Dmel	17	24	0.11 ± 0.43	23.90	82.42	1561.03
Zap2	8	10	0.05 ± 0.25	9.96	16.67	79.12
Dbuzz	1	9	0.04 ± 0.61	-	-	-
Dbusck	2	2	0.01 ± 0.10	-	-	-

† k_{adj} calculated from Taylor et al. (1979)

‡ estimate of *k* of the negative binomial distribution (Bliss and Fisher 1953)

Table 4.3. Estimated k_{HG} (HG method), k_{adj} (Taylor et al. 1979) and k_{NBD} (± S.E.) (Bliss and Fisher 1953) values for each of the identified species for each minimum mapping unit pair. (Dsim= *Drosophila simulans*; Zap1 = *Zaprionus* morphospecies group 1; Dmel = *D. melanogaster*; Zap2 = *Zaprionus* morphospecies group 2; Dbuzz = *D. buzzatii*; Dbusck = *D. busckii*).

Species	Estimated k_{HG}			k_{adj}	k_{NBD} (± S.E.)
	0.04 m^2; 0.16 m^2	0.04 m^2; 0.36 m^2	0.16 m^2; 0.36 m^2		
Dsim	1.162	1.311	1.494	0.432	0.140 ± 0.211
Zap1	0.855	0.683	0.585	0.079	0.058 ± 0.015
Dmel	-	-	-	0.063	0.143 ± 0.076
Zap2	0.618	-	-	0.060	0.097 ± 0.087
Dbuzz	-	-	-	0.062	-
Dbusck	-	-	-	-0.042	-

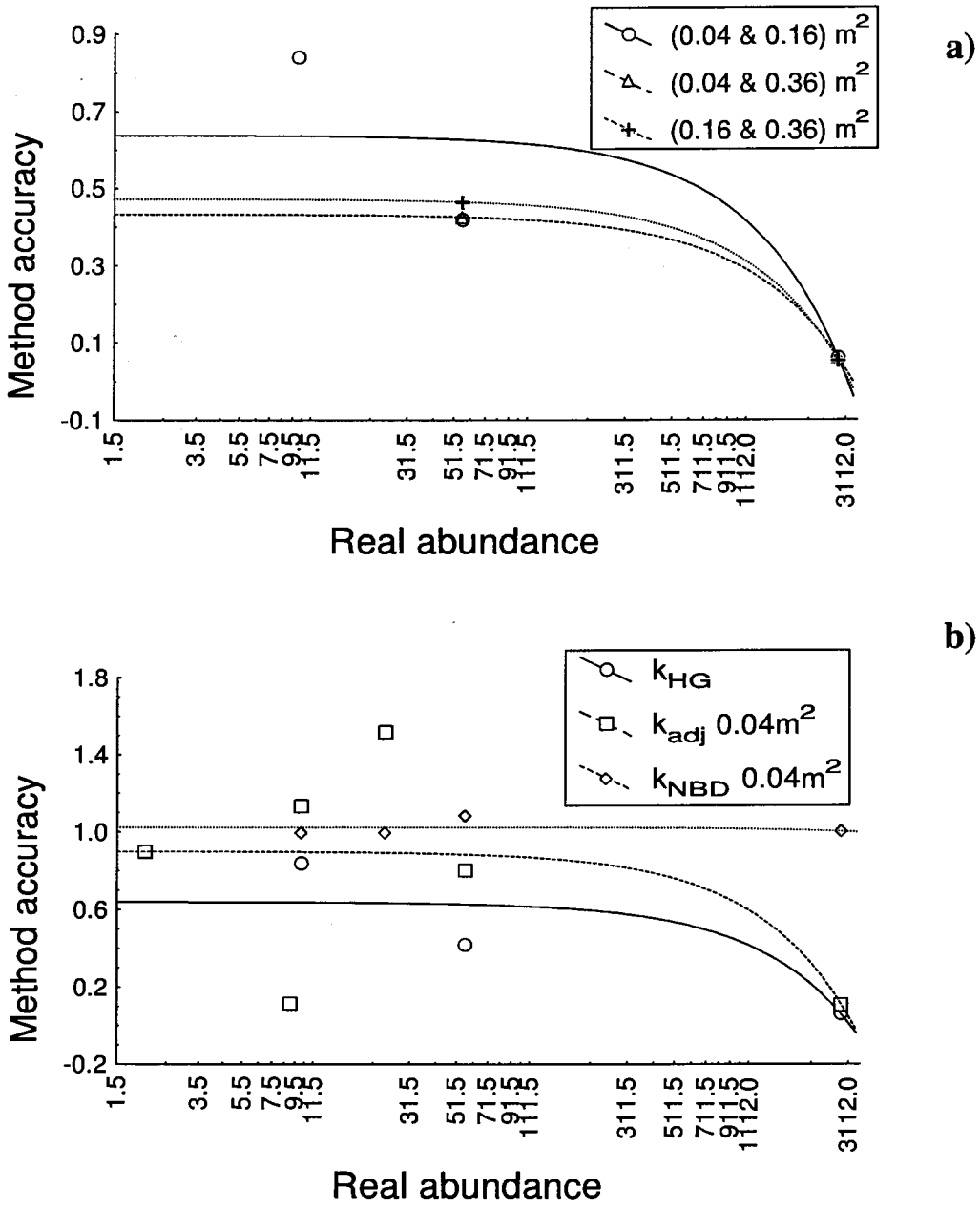

Fig. 4.2. Relationship between method accuracy and real abundance using three different estimates of *k* in the model by He and Gaston (2000a) (HG model): i) k_{HG} as estimated simultaneously with abundance in the HG model; ii) k_{adj} (Taylor et al. 1979); iii) k_{NBD} calculated from the negative binomial distribution (Bliss and Fisher 1953). Relationships are presented for different, and combinations of, three minimum mapping units (MMUs), i.e. 0.04 m^2, 0.16 m^2 and 0.36 m^2. a) Relationships using k_{HG} for the three MMU pairs, i.e. 0.04 m^2 and 0.16 m^2, 0.04 m^2 and 0.36 m^2, 0.16 m^2 and 0.36 m^2. b) Relationships for the three estimates of *k* and MMUs of 0.04 m^2, with k_{HG} calculated using 0.04 m^2 and 0.16 m^2. Method prediction accuracy values closest to 1.0 indicate predicted abundances that are equivalent to measured abundances and are therefore the most accurate. Values below and above one represent under- and over-estimation of abundance respectively.

Discussion

The Drosophilidae assemblage structure found here was very similar to the structure that has been found for other dipteran assemblages associated with ephemeral resources (e.g. Atkinson and Shorrocks 1984, Sevenster and Van Alphen 1993). The high variability in relative abundance between species (*D. simulans* constituted 94 % of all emerged flies) and in occupancy of resources across species appears typical of such assemblages (Beaver 1977, Atkinson 1985, Shorrocks and Rosewell 1987).

The distribution patterns of individuals of each species did not deviate significantly from the NBD. Nonetheless, the HG method consistently underestimated the abundances of species in the assemblage. Estimated k_{HG} values were always larger than both k_{adj} and k_{NBD}. All the species in the assemblage were thus more aggregated than the HG method estimated; k_{HG} lay between 0.58 -- 1.49, while k_{NBD} and k_{adj} ranged between 0.058 -- 0.432 with the lower range of the latter an order of magnitude smaller than k_{HG}. In dipteran assemblages, k is usually less than one, and the k_{adj} and k_{NBD} values calculated here are closer to those reported in the literature than to those estimated as k_{HG} (Atkinson 1985, Shorrocks and Rosewell 1987, Rosewell et al. 1990, Shorrocks, Rosewell and Edwards 1990). The k_{adj} and k_{NBD} values therefore better reflected the highly aggregated nature of fly individuals ($k << 1$), whereas k_{HG} was less sensitive to extreme aggregation. Substitution of k_{NBD} into the HG model thus improved estimated abundances and, at the smallest MMU, the measured abundances of the species were accurately predicted.

Although He and Gaston (2000a) and Kunin et al. (2000) also found that the HG method underestimates abundances, the magnitude of this underestimation was not as large as that found here. In the absence of sampling error, such underestimation is thought to arise in two ways: 1) deviation of the species spatial distribution pattern from the NBD, and 2) MMU's that are too large to detect sufficient variation in species abundance across the sampling extent (He and Gaston 2000a, Falster et al. 2001). The abundance distributions of the Drosophilidae used here did not deviate significantly from the NBD. Rosewell et al. (1990) also found that the NBD adequately described the distribution of *Drosophila* assemblages, and the NBD is not unusual for invertebrate assemblages (Sevenster 1996). However, the distributions of the data used here must deviate to some extent from a perfect NBD. This deviation, although not significant, may be sufficient to result in substantial underestimation of species abundances. Any deviation from the NBD by species that are more strongly aggregated than this distribution predicts, thus results in significant underestimation of abundance values. The limiting values of k lead to other mathematical distributions, and as $k \rightarrow 0$ (strong aggregation) the distribution converges to the

logarithmic series (Quenouille 1949). Therefore, although the maximum likelihood solution approach to calculating k of the NBD is definitive (Anscombe 1950, Bliss and Fisher 1953), the test of significance for the fit of data to a NBD is clearly not sufficiently sensitive to be used to identify assemblages with distributions for which the HG model would be appropriate. Distribution-fitting tests are known to have low power (May 1975, Buzas et al. 1982, McGeoch and Gaston 2002) and are, therefore, not an effective means of determining the appropriateness of the HG model. A statistic more sensitive to departures from the NBD as $k \rightarrow 0$ would be a useful tool for testing the suitability of assemblages for application of the HG model.

A second possible explanation for the underestimation of abundance is the use of minimum mapping units that are too large. In this study the finest possible minimum mapping unit with realistic habitat boundaries was used, i.e. the fruit. Predicted abundances were indeed closest to reality at this smallest MMU for all species. Nonetheless, aggregation still occurred beyond the resolution achievable with this finest MMU, resulting in an overestimation of k by the HG method (k_{HG}). In most highly aggregated assemblages a MMU that is the size of the single resource unit may contain numerous individuals (Atkinson 1985, Shorrocks and Rosewell 1987). Consequently, resource units containing either one or 335 individuals (such as *D. simulans* in this study) will yield identical occurrence maps. A single fruit represents a MMU that is far 'too large' to achieve an accurate estimate of abundance, using the HG method, for assemblages that are highly aggregated. However, it is not only impractical but also unrealistic to use a MMU smaller than a single fruit for assemblages such as this. Therefore, although the use of coarse MMUs is known to reduce the accuracy of abundances predicted (He and Gaston 2000b), even the use of the finest possible, and biologically realistic, MMUs may be inadequate to predict abundances accurately. There are many such assemblages where the resource unit is generally considered the smallest practicable MMU, e.g. those associated with tree holes, dung, carcasses, fruit and flower heads. Although habitat units are usually larger than the size of the individual utilising them, these habitats are i) a standardised unit for all the species in the assemblage (this is required in order to make cross-species comparisons, McGeoch and Gaston 2002), ii) readily measured in the field and iii) represent a habitat with a functional boundary unlike the artificial boundaries of grid cells, i.e. representation-based sample units, *sensu* Gaston (1994b). As a result, habitat units are likely to remain the MMU used for such assemblages.

The HG parameterisation method and its application thus require further exploration at fine scales, often represented by the individual habitat units of species, and for species that are highly aggregated at this scale. Based on published k values (estimated using the NBD) for a

variety of taxa, strong levels of aggregation are apparently widespread; for example, in weevils (Peng and Brewer 1994), eight species of *Drosophila* (Shorrocks and Rosewell 1987, Rosewell et al. 1990), various other Diptera (Atkinson and Shorrocks 1984, Renshaw et al. 1995), eriophyid mites (Hall et al. 1991) and a variety of solanaceous and other, annual, plant species (Johnson et al. 1995, Timmer et al. 1989). Although the k values for these taxa ranged between 7.15 and < 0.005, k values in the range of 0.01 to 0.6 were most common, demonstrating very high levels of aggregation. For species such as these, the only solution for abundance estimation unfortunately remains the use of a reasonably accurate estimate of aggregation (k) prior to application of the model, which requires an *a priori* abundance estimate (e.g. the Nachman model, Nachman 1981, see also Holt et al. 2002a) and thus defeats the purpose of the He and Gaston (2000a) approach.

There are obvious advantages to being able to apply the HG model under conditions of strong aggregation at fine scales. For example, scouting for pest populations in fields, orchards and vineyards results in presence-absence (occupancy) data for crop units, such as trees, leaves or fruit (Overholt et al. 1994, Wilson and Morton 1993). Were it possible to derive accurate predictions of pest abundance from these data alone, more reliable damage estimates and economic injury levels could be calculated. Other than the insensitivity of the parameterisation method to very high degrees of aggregation, the HG model appears highly suitable for such application. Pest abundance estimates are generally required for scales in the order of single or multiple fields or orchards, scales much finer than geographic mapping data readily available for many plant and vertebrate taxa. Mapping units and sampling extent are thus comparatively fine. Furthermore, predictions of abundance and k could be derived using a short range of scales (small spatial scale differences between the two minimum mapping units used), which is another of the conditions for optimal performance of the HG method (He and Gaston 2000b, Kunin et al. 2000).

A possible future solution for improving abundance predictions for highly aggregated, abundant species may involve the incorporation of information on the relative spatial positions of occupancy measures, i.e. the degree to which nearby points have the same state (occupied or unoccupied) compared to distant points. Here, the distinction is made between aggregation in the form of statistical versus spatial heterogeneity (Perry 1998). Statistical heterogeneity arises from kurtosis in the frequency distribution of records, and the degree to which this fits a particular distribution (such as the NBD), whereas spatial heterogeneity results from the clumping of records, or their deviation from a regular spatial arrangement (Perry 1998). Aggregation

estimates based on k of the NBD, including those estimated by the HG method, quantify statistical heterogeneity, but do not consider the spatial heterogeneity of abundance-occupancy records. However, for abundant species, deviation from spatial regularity is likely to be large when the level of aggregation by individuals within habitat units (or MMUs) is high. Indeed, the abundances of rare species in this Drosophilidae assemblage were more accurately predicted by the HG method than the abundance of the common species (although the HG model performs equally well for rare and common species when provided with an accurate estimate of k). Therefore, for species known to be abundant (e.g. eruptive or outbreak species), estimates may be improved by incorporating this additional dimension of spatial information into abundance-occupancy models (see approaches by Perry 1998, Perry et al. 1999). Such spatially explicit exploration of the approach pioneered by He and Gaston (2002a) would clearly repay the effort because the latter provides a useful and much-needed means of predicting abundance from occupancy data, with considerable value in applied ecology.

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CHAPTER 5

Body size patterns in *Drosophila* inhabiting a mesocosm: interactive effects of spatial variation in temperature and abundance

Introduction

Body size is a life history variable of considerable significance. It has a major effect on other traits, such as physiological rates, fecundity, longevity, and competitive ability (Schmidt-Nielsen 1984, Roff 1992, 2002, Stearns 1992, Krijger et al. 2001), and therefore influences both the rate of population increase and abundance (Nunney 1996, Brown and Gillooly 2003, Cohen et al. 2003). In other words, it is a major component of fitness. Size also affects population energy use (Brown and Maurer 1986, Blackburn and Gaston 1996, Brown and Gillooly 2003), and is intimately related to spatial variation in species richness (Siemann et al. 1996, 1999, Allen et al. 2002, Ulrich 2004). In consequence, much attention has been given not only to the mechanisms which underlie the influence of body size on these variables, but also to how life history traits and other factors interact to determine size at maturity, and its change thereafter in long-lived organisms (e.g. Roff 1981, 1992, 2002, Kozłowski 1996, 2002, Benton and Grant 1996, Kozłowski and Teriokhin 1999, Blanckenhorn 2000, Gotthard 2004, Kozłowski et al. 2004).

It is now theoretically well established that resource availability and quality, competition, the likelihood of mortality (especially in juveniles), production rate, and the length of the growing season all contribute to optimal (≈ fitness maximising) size at first reproduction (see reviews in Roff 2002, Kozłowski et al. 2004). However, the relative contributions of these factors to optimal size (and subsequent size increases in species with indeterminate growth) have not been fully established empirically (Blanckenhorn 2000, Angilletta and Dunham 2003, Angilletta et al. 2004a). In particular, the determinants of spatial and temporal variation in size are poorly investigated (Chown and Gaston 1999, Kari and Huey 2000, Blanckenhorn and Demont 2004). One exception is *Drosophila* species. Considerable empirical work has been done on the proximate and ultimate determinants of body size, both in the laboratory and in the field, using *Drosophila* species as model organisms.

In the laboratory, the effects on body size of various factors, such as temperature (Partridge et al. 1994, Pétavy et al. 2001), larval crowding (Delcour and Lints 1966, Santos et al. 1994), temperature and food concentration (De Moed et al. 1997), ethanol concentration (Hageman et al. 1990, Elamrani and Idaomar 2000), and desiccation (Hercus and Hoffmann

1999, Gibbs and Matzkin 2001), have been widely investigated. This work has regularly included examinations of the proximate determinants of size variation (e.g. changes in cell size vs. cell number – see Partridge et al. 1994, Partridge and Coyne 1997, French et al. 1998, Azevedo et al. 2002). Laboratory investigations of downstream effects of size variation on life history traits such as survival (McCabe and Partridge 1997), fecundity (McCabe and Partridge 1997, Nunney and Cheung 1997, Yenisetti and Hegde 2003), male reproductive success (Bangham et al. 2002, Yenisetti and Hegde 2003), and longevity (Zwaan et al. 1992, James and Partridge 1995) have also been performed. Field investigations of patterns in and the likely mechanistic determinants of spatial and temporal size variation have revealed that factors similar to those identified in the laboratory underpin size variation. These include spatial and seasonal variation in temperature, variation in resource and water availability, and abundance effects via crowding and resource appropriation or alteration (e.g. Stalker and Carson 1948, Levins 1969, Atkinson 1979, Barker 1983, Coyne and Beecham 1987, Thomas 1993, Worthen et al. 1993, Worthen et al. 1994, James and Partridge 1995, 1998, Worthen 1996, Borash et al. 1998, Karan and Parkash 1998, Huey et al. 2000, Jenkins and Hoffmann 2000, Kari and Huey 2000).

Despite substantial recent progress in reconciling laboratory and field findings, several difficulties stand in the way of integrating them (e.g. Weeks et al. 2002). For example, it is clear that laboratory and field flies differ in several ways, including size (Moreteau et al. 1995, David et al. 1997, Gibert et al. 1998, Jenkins and Hoffmann 2000), longevity (Boulétreau 1978), and the responsiveness of size to directional selection (Gibbs and Matzkin 2001). Whilst laboratory studies generally, and rightly, hold all factors constant, whilst examining variation in the one of interest, they offer model organisms an environment very different to the one they are likely to experience naturally. This in turn might make the findings of laboratory studies incompatible with the situation in the field. For example, *D. melanogaster* evolves increased water content under desiccation in the laboratory, but this trait is not typical of xeric vs. mesic species, probably because of manoeuvrability problems associated with larger size (Gibbs et al. 1997, Gibbs and Matzkin 2001). By contrast, field studies, and especially those undertaken over large spatial and temporal scales, have to contend with multiple interacting factors, such as water and resource availability, temperature, day length, parasitism, and abundance (Borash et al. 1998, Houle and Rowe 2003). For example, whilst rapid development is likely to increase fitness by decreasing the time that eggs and larvae are exposed to parasitoids and declining resource quality (James and Partridge 1995), it might also lead to a decrease in body size and therefore greater susceptibility to starvation (Chippindale et al. 1996). Likewise although high temperatures

induce developmental problems (which affect size, fecundity and survival) (Feder 1997, Feder et al. 1997a, Feder and Krebs 1998), they simultaneously effect a decline in population density for survivors. This might result in lower intra- and inter-specific competition between surviving individuals that are able to behaviourally avoid temperature stress (i.e. able to locate a thermal refuge (Wilmer 1982, Feder 1997)). Individuals developing in more suitable conditions might grow larger than expected under greater densities (Worthen et al. 1994). Thus even when field patterns seem to reflect those found in the laboratory, and correlative studies reveal potentially similar mechanisms, ascertaining the causal factor(s) underlying size variation remains problematic. In consequence, there have been several recent calls for investigations into the interactions between mechanisms likely to affect life history variables, such as body size, under controlled field conditions (Jenkins and Hoffmann 2000, Pétavy et al. 2001, Gibbs 2002, Hoffmann et al. 2003a, see also Angilletta et al. 2004b). One effective way of combining the control of laboratory studies with the more realistic conditions of the field is by using a mesocosm approach (Warren and Gaston 1997, Petersen and Hastings 2001, Relyea and Yurewicz 2002, Srivastava et al. 2004). Here, factors of interest can be intentionally manipulated in a controlled fashion whilst others remain a function of the "natural" environment.

In this study a mesocosm experiment, consisting of a regular lattice of nectarine fruit exposed to sun or shade (see Warren et al. 2003), is used to investigate the influence of spatial variation in temperature on adult body size in *Drosophila simulans* Sturtevant. Spatial variation in resource quantity and other abiotic variables (e.g. relative humidity and water availability) is effectively constant because they show the same natural temporal variability across the spatial treatment. Moreover, rather than fixing abundance per resource unit, this is allowed to vary, but is measured, so enabling us to investigate interactions between abundance (larval crowding) and temperature on final body size. Based on what is known of the effects of both temperature and abundance on adult body size in *Drosophila* spp. the following predictions were made. In the absence of interactive effects, larger adult flies are expected to emerge from shaded fruit, owing to the effects of temperature on size (see Atkinson 1994, David et al. 1997 for review). Similarly, flies in unshaded fruit might also be characterised by higher levels of developmental abnormality and smaller body size if unshaded fruit represent a stressful environment owing to high temperature (see Feder 1997, Feder et al. 1997a, Feder and Krebs 1998). The typical response in *Drosophila* spp. to high temperature is expression of heat shock proteins (Krebs and Feder 1998), or their diversion from normal developmental regulation (Rutherford and Lindquist 1998), for protein chaperone purposes. Both of these processes interfere with normal growth and

development (review in Chown and Nicolson 2004), and would result in smaller body size and a high incidence of developmental abnormalities (see Roberts and Feder 1999) in the unshaded treatments. The end result of the action of the main effects hypothesis is therefore that larger flies develop in the shaded treatments, while stressfully high temperatures in the unshaded treatments induces developmental abnormalities and smaller flies. However, the outcome of body size may not be cut-and-dried, i.e. there may well be interactive effects on the outcome of body size. Mortality associated with temperature stress in unshaded fruit might mean a lower larval density than in shaded fruit and therefore relaxed crowding (leading to greater resource availability and quality). Larvae that are able to find thermal refuges in unshaded fruit might then be capable of reaching a large body size because of improved resources compared with shaded fruit. This does not mean that the compensation of the outcome of temperature and crowding on body size is likely to be equal. Namely, a continuum of body sizes where one or both processes are affecting size to a differing degree is the more likely scenario. Establishing that interactive processes affect body sizes in natural assemblages facilitates the reconciliation of results obtained from laboratory and field studies. Distinguishing the simple from interactive predictions is relatively straightforward in terms of size patterns. In the latter case there should be little spatial pattern in body size associated with shade and sun patches, whereas in the former case spatial association should be strong. However, understanding the likely mechanisms underlying the pattern requires more complex analysis. Here both are provided.

Materials and Methods

Experimental design and sampling procedure

A Drosophilidae assemblage was allowed to naturally colonise a mesocosm (hereafter the 'study arena') comprising decaying nectarines (*Prunus persicae* Miller variety *nectarina*: Rosaceae) (see Warren et al. 2003). The study arena was divided into six plots and placed out, on a wire table (2.2 m x 3.4 m; 0.7m high), at the University of Pretoria's Experimental Farm in Pretoria, South Africa (25°45.178"S, 28°15.293"E). Three of the plots in alternate rows of the two columns were artificially shaded (15 cm above the fruit) with 80 % shade netting to impose variation in the microclimate to which the fruit and therefore the fly larvae were exposed, i.e. three shaded plots and three plots exposed to the sun (Fig. 5.1). Each plot contained 36 nectarines spaced 20 cm apart in a regular grid of 12 x 18 fruit, each on a coarse plastic mesh in the centre of a round plastic container (~ 15 cm diameter and 8 cm deep) containing washed, moist sand. The grid therefore resembled an arrangement of fruit under neighbouring trees in an

orchard albeit more regular than what would usually occur naturally. The table was placed inside a wire-covered cage to exclude birds, fruit-piercing moths and large wasps (which may influence resource quality and quantity). Ants were excluded by the application of grease to the table legs. Nectarines were washed and weighed to estimate the resource quantity per fruit, before being placed in the field. Initial fruit mass did not vary between treatments (Sun: Mean ± S.E. = 58.59 g ± 1.09; Shade: Mean ± S.E. = 59.08 g ± 1.07; $t = 0.35$, $df = 214$, $P = 0.72$). Therefore, resource quantity available to the flies at the start of the experiment was similar for both treatments.

Three small puncture holes were haphazardly made in the skin of the fruit before placement in the field because *Drosophila* species do not lay eggs on unbroken fruit surfaces (Atkinson 1983, Feder and Krebs 1998). Five nectarines were randomly selected for insecticide residue tests and were found to have no detectable levels of residues of the following chemicals used in the local soft fruit industry: organophosphates (Dimethonate, Malathion, Triazophos), organochlorides (γ-BHC, β-Endosulphan, Endosulphan sulphate) and pyrethroids (Cypermethrin, Deltamethrin) (South African Bureau of Standards).

Six copper-constantan thermocouples were placed 1 cm deep under the skin of six nectarines to measure fruit temperature. Three nectarines in one of the shaded plots and three nectarines in one of the exposed (sun) plots were selected (from the edge of a treatment plot to the interior) to represent the range of temperatures experienced by the flies occupying the fruit (Fig. 5.1). Temperature measurements were taken every 10 minutes for the duration of the experiment. The mean vapour pressure deficit for each day (mean VPD) was calculated for the study arena using a non-aspirated psychrometer. VPD is a better measure of desiccation stress than relative humidity for small insects (Unwin and Corbet 1991) and has been shown to be correlated with egg and pupal mortality in *D. melanogaster* (Al-Saffar et al. 1995). Although rainfall (mm per day) was recorded using a tipping-bucket rain gauge at the site, rainfall was reasonably highly correlated with mean VPD ($r_s = -0.77$, $P < 0.05$), as may be expected, and rainfall was excluded in all analyses of the full dataset. Wind speed was recorded using a R.M. Young wind sensor and solar radiation was measured with a LiCor silicon pyranometer. All data were recorded (at 10 minute intervals) by a Campbell Scientific CR10 data logger using PC208 software for programming and data capture. The experiment ran for 25 days in November 1998.

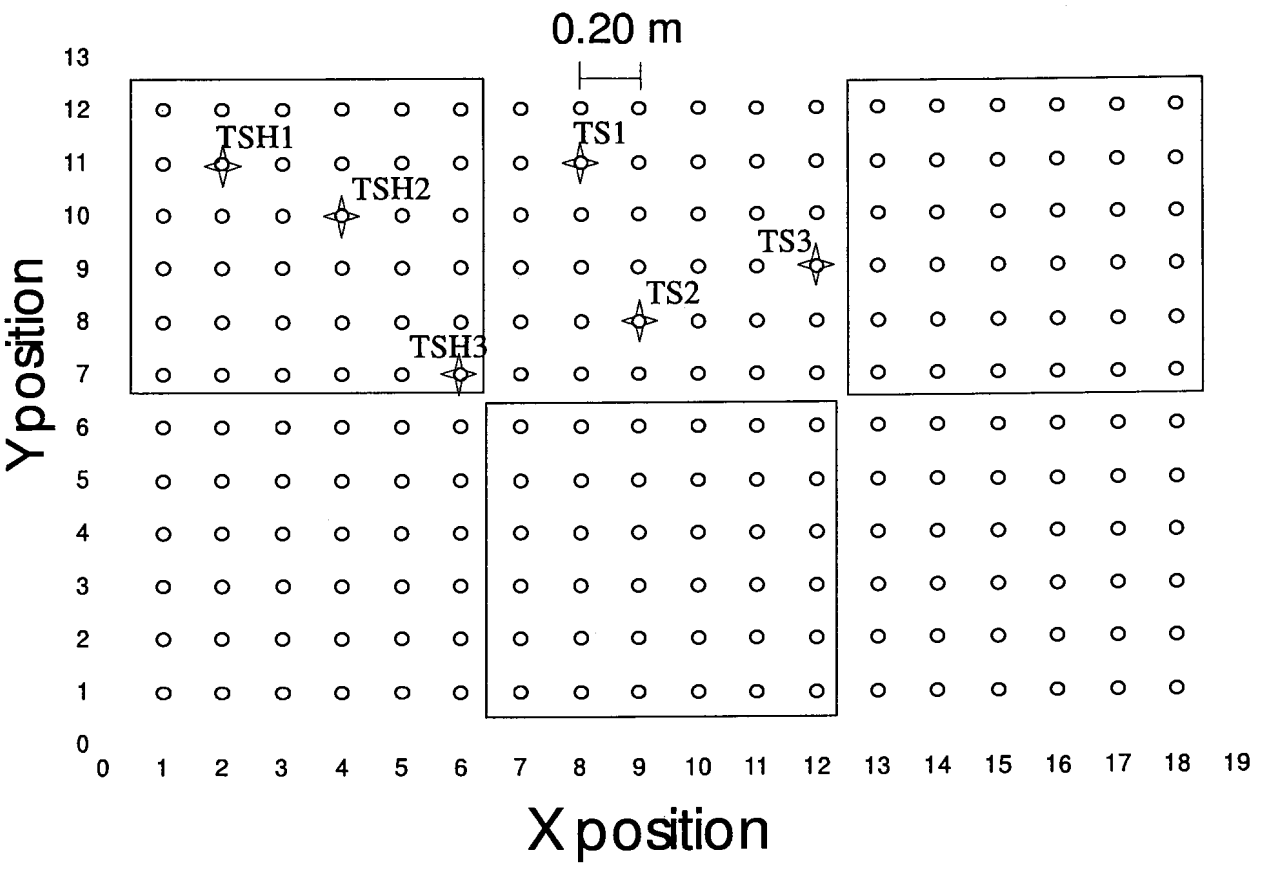

Fig. 5.1. Plot layout depicting thermocouple positions (stars) in the shaded (TSH1 – TSH3) and sun (TS1 – TS3) fruit (circles). Thin solid outlines around groups of fruit represent plots that were shaded with 80 % shade netting.

Because temperature changes during larval development may influence the adults that finally emerge from the fruit, the nectarines remained in the field for the duration of the experiment. At the pupal stage, the insect has already consumed the food required to become an adult. Thus removal of the pupae from the field will not influence the linear dimensions or species composition of the emerging adults. Pupation was expected to take between 4.5 and 8.5 days for flies at 25 °C and 80 % RH (Sevenster and Van Alphen 1993). Every second day for 25 days, starting from the fifth day after laying out the experiment, the sand under the nectarines containing the fly pupae was removed and placed in 350 ml jars. This was repeated eleven times (sampling day one to eleven). Fresh, moist sand was placed then in the containers under the fruit. The sand was kept moist by spraying a standard volume of water onto the sand each day. The

jars were then taken to the laboratory and the emerging flies were recorded, identified (according to McEvey et al. 1988) and measured. At least six Drosophilidae species were found but specimens belonging to the genus *Zaprionus* could not be identified to species level. The identified species were: *Drosophila simulans* Sturtevant, *D. melanogaster* Meigen, *D. busckii* Coquillett, *D. buzzatii* Patterson and Wheeler, *Zaprionus* morphospecies group 1 (*Zaprionus* msg 1) (may include both *Z. tuberculatus* Malloch and *Z. sepsoides* Duda) and *Zaprionus* morphospecies group 2 (*Zaprionus* msg 2) (may include both *Z. vittiger* Coquillett and *Z. indianus* Gupta) (McEvey et al. 1988). However, because *D. simulans* dominated all samples (96 % of all the measured flies were of this species), only *D. simulans* was investigated.

Data

Thorax length was measured (to the nearest 0.01 mm), as the distance from the anterior margin of the thorax to the posterior tip of the scutellum as viewed from the side, with a binocular microscope fitted with an ocular micrometer. Thorax length is positively correlated with body size and this was taken as an estimate of body size (Robertson and Reeve 1952, Cowley and Atchley 1990). Because body size varies with sex (females are larger than males, Partridge et al. 1994, Santos et al. 1994, Crill et al. 1996) all flies were sexed. The presence of abnormally developed wings (one or two) was taken as an indication of development under stressful conditions. The flies were scored as either W0: no wing abnormality, normal wings; W1: slight curling of one or both wings; W2: severe curling of one or both wings (abnormalities appeared similar to those recorded by Roberts and Feder (1999)). As a result of thorax and abdomen damage, only 6847 of 7228 *D. simulans* individuals were measured and sexed and used in the analyses below.

No flies and two flies were recorded for sampling days one and two, respectively, and these days were therefore excluded from the analyses. All analyses were performed for the full dataset (sampling days 3-11) and for sampling days 9 and 10 (low and highest abundances) separately, to exclude possible successional (sampling day) effects while examining the influence of abundance on body size.

Analytical approach

To demonstrate that sun and shade treatments differed with respect to temperature, means, minima, maxima and ranges were calculated for each thermocouple across the experimental time period. Differences in thermocouple temperature measures between the two plot types were

tested using Mann-Whitney U tests (Zar 1984). Differences in the time (number of hours) that fruit in the sun and shade were exposed to temperatures above 32 °C (Hsp induction takes place above 32 °C, Feder et al. 1997a), and above 37 °C (lethal for drosophilids, Feder and Krebs 1998) were assessed using Mann-Whitney U tests (Zar 1984).

The relationships between sampling day, sex, wing abnormality (normal or abnormal, i.e. a collapse of categories W1 and W2 into one), mean VPD per day, treatment (sun or shade), fruit mass and total abundance were examined using Spearman's rank correlation coefficients for the full dataset (sampling days 3 – 11) and sampling days 9 and 10 (Zar 1984). In general, although some terms were significantly correlated with one another ($r_s < 0.70$), tolerances were high (> 0.83) indicating that collinearity was unlikely to severely influence the outcomes of the generalised linear models (Quinn and Keough 2002).

One of the primary predictions of the interactive vs. non-interactive hypothesis was that the pattern of body size variation across the study arena would show little or no spatial structure in the former case. Spatial pattern in thorax length (sum of all thorax lengths and mean thorax length per fruit) was investigated using spatial autocorrelation (Moran's *I* and omnidirectional correlograms) (SAAP v 4.3, Wartenberg 1989). 15 distance classes were chosen with equal distance intervals (equivalent to 0.27 m) and therefore the number of point pairs varied with distance class. Distance classes with fewer than 1 % of the total number of point pairs in a class should not be interpreted and the correlograms were therefore only drawn for distance classes one to ten (Legendre and Fortin 1989). The data were transformed (ln) to stabilise the variance prior to analysis (Legendre and Legendre 1998). In the absence of interactive effects but in the presence of a simple effect of temperature on size, certain predictions may be made regarding thorax length correlogram structure. If the thorax lengths of flies developing in shaded treatments were larger than those developing in unshaded (sun) treatments, then the correlograms of thorax length should be positively autocorrelated for distances 0.00-1.08 m and 2.3-2.7 m and negatively autocorrelated for distances 1.08-2.3 m. In square sampling areas, the x-intercept (where the correlogram becomes negative or zero) approximates the length of one side of the square (Sokal and Wartenberg 1983) and should therefore approximate six fruit neighbours for this experimental design. The first four distance classes correspond to the size of a microclimate treatment plot (six fruit neighbours). Distances 1.08-2.3 m correspond to the distance between adjacent plots (7--12 fruit neighbours), while the distances 2.3-2.7 m correspond to the distances between adjacent plots of the same treatment (13--18 fruit neighbours).

Spatial patterns in wing abnormalities were investigated using Spatial Analysis by Distance IndicEs (SADIE) (Perry et al. 1996, Perry 1998). SADIE explicitly incorporates spatial information associated with samples (localities) into the quantification of spatial pattern, and was used to determine the degree of aggregation in wing abnormality categories across the study arena, as well as to examine the extent of spatial aggregation at individual localities (fruit) (Perry 1995, 1998, Perry et al. 1996, 1999). SADIE assigns a sample an index of aggregation (I_a), and probability of aggregation (P_a), by comparing the spatial arrangement of the observed distance to regularity with the permuted distances to regularity derived from a randomisation procedure (Perry 1995). Values of I_a greater than 1.0 indicate spatial aggregation, those approximating 1.0 indicate randomness, and those less than 1.0 indicate regularity (Perry 1995).

SADIE was also used to relate the recorded number of normal winged flies (W0) and flies with severe wing abnormalities (W2) at an individual fruit locality to the environmental surface at the same locality (Perry et al. 1996, Perry 1998). Spatial pattern in flies with slight wing abnormalities (W1) were not investigated using SADIE as a result of the low number of flies (n = 80) recorded for this category and the few fruit occupied by these flies. Under these conditions SADIE results may suffer from high Type II error rates, i.e. lack of power because of low occurrence rates (Korie et al. 2000). SADIE was used to determine the degree of aggregation in wing abnormality categories across the study arena, as well as to examine spatial aggregation at individual localities (fruit). SADIE is also able to identify patches (v_i >1.5; areas of high counts) and gaps (v_j <-1.5; areas of low counts) (Perry et al. 1999). The v_i and v_j values for each fruit for wing abnormality categories were plotted to visually inspect clustering across the study arena (Perry et al. 1999). The average patch ($\bar{v}_i$) and gap ($\bar{v}_j$) distances were calculated to formally test for overall clustering in wing abnormality counts across the study arena. Xu and Madden (2003) show that the magnitude of SADIE patches and gaps are dependent on their distance from the centre of the study arena. However, the effect is unlikely when more than two patches are present. Here, the study arena consists of six plots (three are likely to contribute to patches and three to gaps) and the arena is held constant throughout sampling. In addition, the centre of the arena lies on the boundary between a sun and shade plot, and because of the experimental layout (Fig. 5.1) additional sun and shade plots lie at equal distances on either side of the centre point. Furthermore, I_a, $\bar{v}_i$ and $\bar{v}_j$ values were not compared across studies, rather the presence of significant patches and gaps was determined and where these are located relative to the imposed microclimatic treatment plots. Therefore, the criticism concerning the magnitude of patches and

gaps raised by Xu and Madden (2003) are unlikely to bias the conclusions drawn from the SADIE results (see also Veldtman and McGeoch 2004).

Subsequent to these analyses, generalised linear models (GLZ) with normal error structure and identity link function were used to evaluate differences in *D. simulans* thorax length (dependent variable) between explanatory variables for the full dataset, and for sampling days 9 and 10 separately (STATISTICA v5.5, Statsoft 2000, McCullagh and Nelder 1989). The best subsets likelihood ratio approach was used to determine the best-fit model with fewest terms in which the likelihood ratio statistic did not change significantly for one degree of freedom (McCullagh and Nelder 1989, Collet 1991, Dobson 2002). The goodness of fit was measured using the deviance statistic (deviance/df close to one indicates a good fit) and the percentage deviance explained (% de) for the best fitting model (for each data subset) was calculated (McCullagh and Nelder 1989, Collet 1991, Dobson 2002). The change in deviance for single variables in the final model was used to estimate the contribution of individual variables to the total explained deviance by the final model (Collet 1991, Lobo et al. 2002). The above analyses were repeated with cumulative abundance in the analyses of sampling days 9 and 10 (summed abundance up to and including sampling day 9 and sampling day 10 respectively) substituted for total abundance. Cumulative abundance represents the number of larvae that have utilised a fruit from the time of placement in the field to the sampling day of interest. High cumulative abundance may adversely affect body size by decreasing resource quantity and/or quality, and/or by increasing competition (e.g. increased toxicity) (Barker 1983, Hageman et al. 1990, Borash et al. 1998).

An additional Type III sums of squares GLZ with Poisson error structure and log link function was used to evaluate differences in the abundance of abnormally winged flies (dependent variable) between explanatory variables for sampling days 9 to 11 (STATISTICA v5.5, Statsoft 2000, McCullagh and Nelder 1989). The effects of temperature and changing resource quality over time were accounted for by including treatment and sampling day as terms in the analysis while controlling for larval crowding (total abundance of flies per fruit). If treatment is important for explaining the proportion of abnormally winged flies, then this term should be significant. If the proportion of abnormally winged flies changes through time, then sampling day should be significant too.

Results

The prevailing abiotic conditions over the course of the study were (Mean ± S.E.) wind speed: 1.01 ± 0.80 $m.s^{-1}$, solar radiation: 69.73 ± 2.00 $W.m^{-2}.day^{-1}$ and a total of 102.36 mm rainfall on 10 days (see Fig. 5.2). The highest total rainfall (35.81 mm) and mean wind speeds (2.12 ± 0.08 $m.s^{-1}$) per day, and lowest mean solar radiation (6.66 ± 0.81 $W.m^{-2}$) per day were recorded on sampling day 5. All measured abiotic conditions were relatively stable from sampling days 7 to 11 (Fig. 5.2). Mean VPD prior to sampling day 5 was often higher than for the remaining sampling days (Fig. 5.2c). The VPD dropped drastically at sampling day 5 and restabilised fluctuating around a value of 0.7 kPa until the completion of the experiment (Fig. 5.2c). A clear successional pattern in fruit decomposition was observed from initial placement of the fruit in the field to the final (11th) sampling day. Fruit developed brown necrotic patches, became soft and by sampling day 5 were drying out rapidly. At the conclusion of the experiment, the fruit were shrivelled, dry and most were black in colour. Temperatures were significantly different between sun and shade plots, with the temperature range in the sun 10 °C greater than in the shade (Table 5.1). Daily temperature regimes of the fruit in the sun and shade plots were similar except that the maximum temperatures achieved in the sun were significantly higher than those measured in the shade (Table 5.1). More importantly, the time that fruit were exposed to temperatures above 32 °C and above 37 °C was significantly longer for the sun than for the shade treatments (Mann Whitney U tests results were the same for temperatures above 32 °C and above 37 °C, $U = 0.00$, $Z = 1.96$, $P < 0.05$, Fig. 5.3). Therefore, the microclimatic treatment resulted in substantial differences in the thermal environments between sun and shaded treatments.

Initial colonisation of the fruit by drosophilids was weak and the abundance of emerging flies was low for sampling days 3 - 7 (Fig. 5.4). The abundance of emerging flies was much higher on sampling days 8 - 11 with the highest abundance recorded for sampling day 10 (Fig. 5.4). Furthermore, as the resource aged, emerging flies decreased in size from sampling days 7-11 (Fig. 5.4).

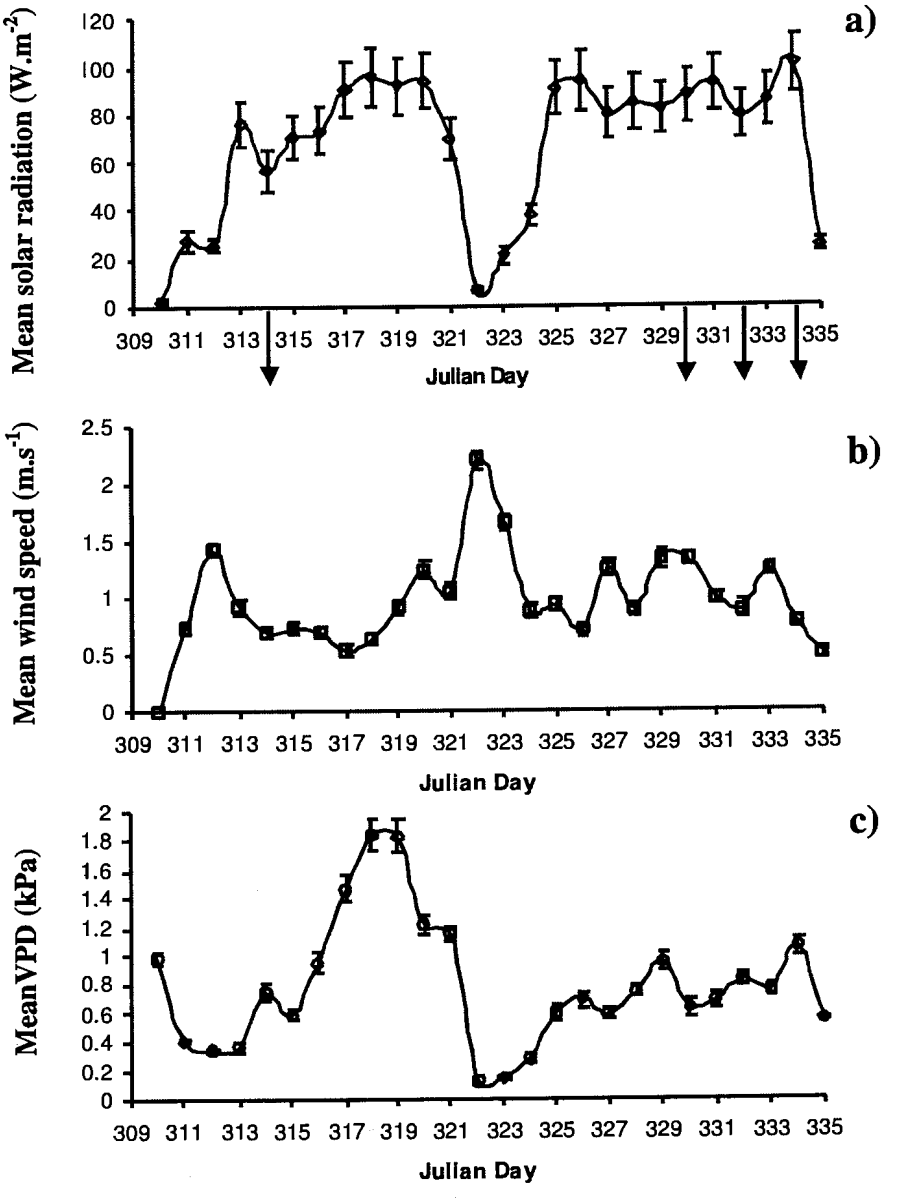

Fig. 5.2. a) Mean (± S.E.) solar radiation, b) wind speed and c) vapour pressure deficit (VPD) over the temporal extent of the study. Arrows depict sampling days 1, 9, 10 and 11 respectively.

Table 5.1. Temperature (°C) means, minima, maxima and ranges for fruit in the sun (n = 3) and shade (n = 3).

	Sun plots	Shade plots	U	Z	P <
Mean (± S.E.)	20.67 ± 0.09	18.82 ± 0.06	0.00	1.96	0.05
Minimum	6.37	8.19	2.00	-1.09	0.28
Maximum	54.11	46.06	0.00	1.96	0.05
Range	47.73	37.87	0.00	1.96	0.05

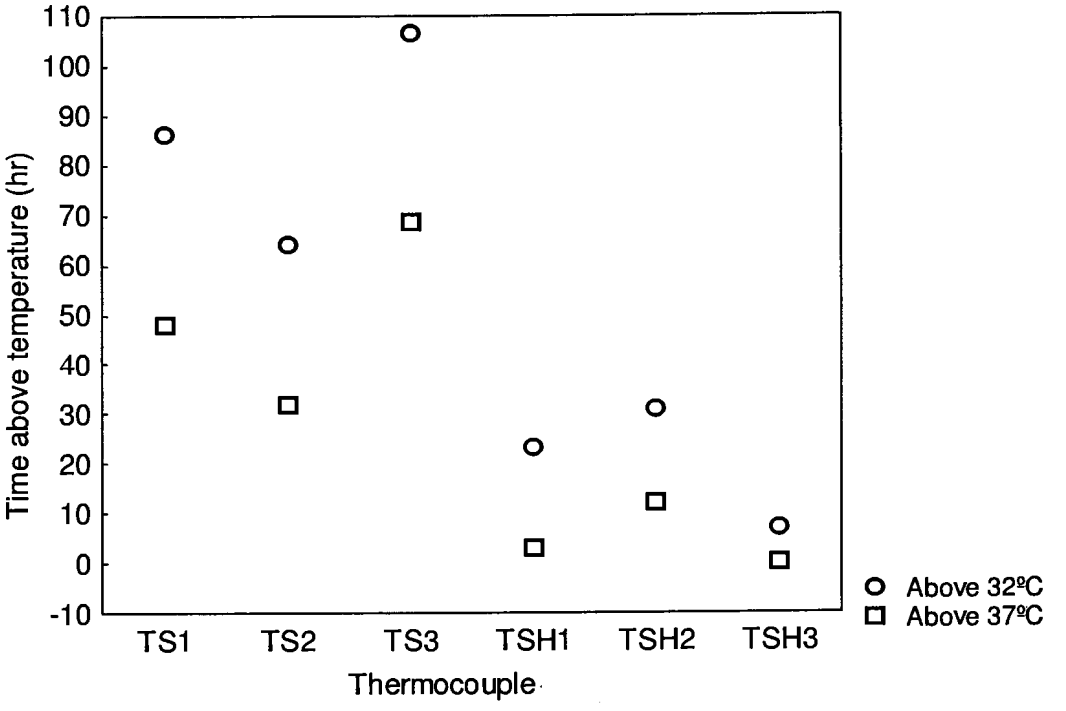

Fig. 5.3. Thermocouple readings for the number of hours recorded above 32 °C and 37 °C for thermocouples in the sun (TS1, TS2, TS3) and shade (TSH1, TSH2, TSH3) positions.

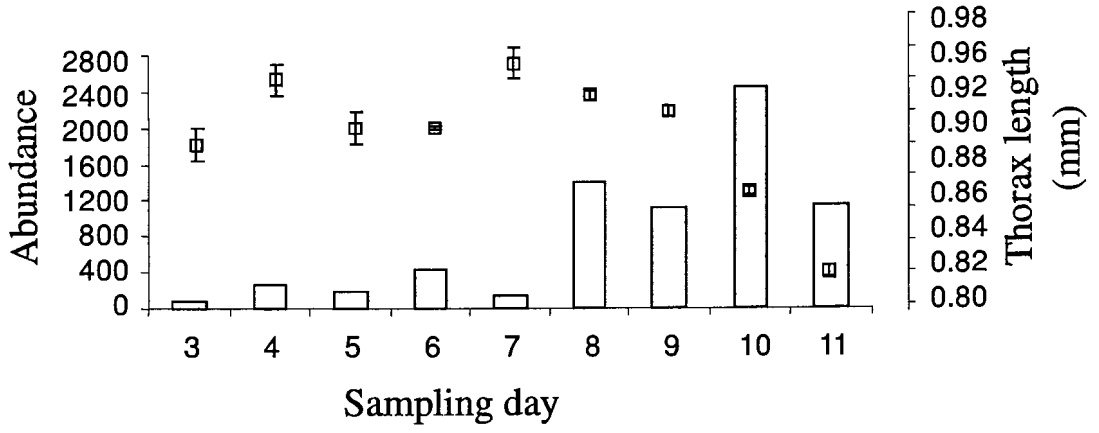

Fig. 5.4. Abundance of measured flies (bars) and mean (± S.E.) thorax length (squares) for each sampling day.

Sampling days 3 – 11

A spatial map of the sum of thorax lengths per fruit (so including abundance) was spatially structured with higher values for those plots in the shade, while that of mean thorax length per fruit (i.e. taking sampling day into account) did not display much structuring (Fig. 5.5a). The spatial autocorrelation analyses confirmed this (Fig. 5.5b). Although the structuring present in sum of thorax lengths matched the expectation if the non-interactive effect of treatment (temperature) was structuring thorax length (Fig. 5.5a,b, x-intercept between distances 0.81-1.08 m approximating the length of one side of the square treatment plots), taking abundance into account removed this significant spatial structuring (Fig. 5.5b). Therefore, the hypothesis of interactive mechanisms determining the outcome of thorax length is supported by the autocorrelation analysis as no spatial structure in present in mean thorax length.

The SADIE analysis revealed that wing abnormality scores were significantly aggregated (Table 5.2). Aggregation was strongest for the flies displaying no abnormalities (W0), and patch and gap indices were largest and most significant for these flies (Table 5.2). Clusters of patches and gaps were identified for the severest wing abnormalities recorded (W2, Table 5.2). Although normal winged flies clustered into patches in the shaded treatments (Fig. 5.6a), patch clusters of the severest wing abnormality class also occurred exclusively in shaded treatments (Fig. 5.6b). Thus, it appears that for surviving larvae, developmental conditions were not as stressful in the sun as in the shade treatments.

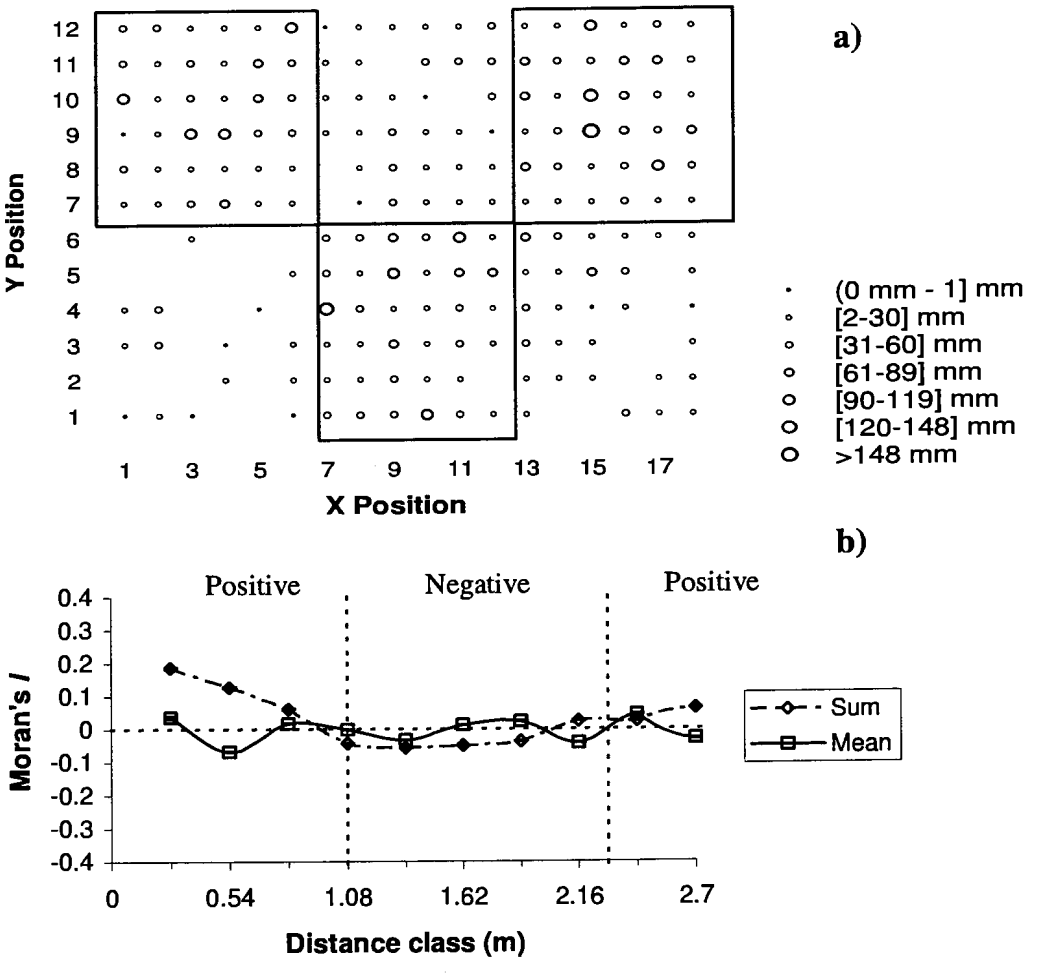

Fig. 5.5. Spatial pattern in thorax length. a) Map of sum of thorax length (mm) of all flies emerging from each fruit, and b) Omnidirectional correlogram of sum and mean thorax length across distance classes (Bonferroni corrected overall correlogram significance levels $\alpha = 0.001$ and 0.292). Vertical lines correspond to sections of correlogram predicted to be positive and negative respectively (see text for details). Filled symbols indicated significant autocorrelation for that distance class. Outlined blocks in a) represent plots that were shaded.

Table 5.2. Aggregation indices and the number and size (represented by number of fruit in brackets) of gap and patch clusters of wing abnormality categories (W0 = no abnormality, W2 = severe wing abnormality) for the pooled data. I_a = Index of patchiness, $\bar{v}_j$ = the average value of v_j over all inflows, $\bar{v}_i$ = the average value of v_i over all outflows, gap = area of low counts, patch = area of high counts. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Data	n	I_a	$\bar{v}_j$ (Gaps)	$\bar{v}_i$ (Patches)
W0	6340	2.141***	-2.016***	2.122***
			(8, 83)	(7, 50)
W2	427	1.490**	-1.466*	1.55**
			(7, 53)	(11, 26)

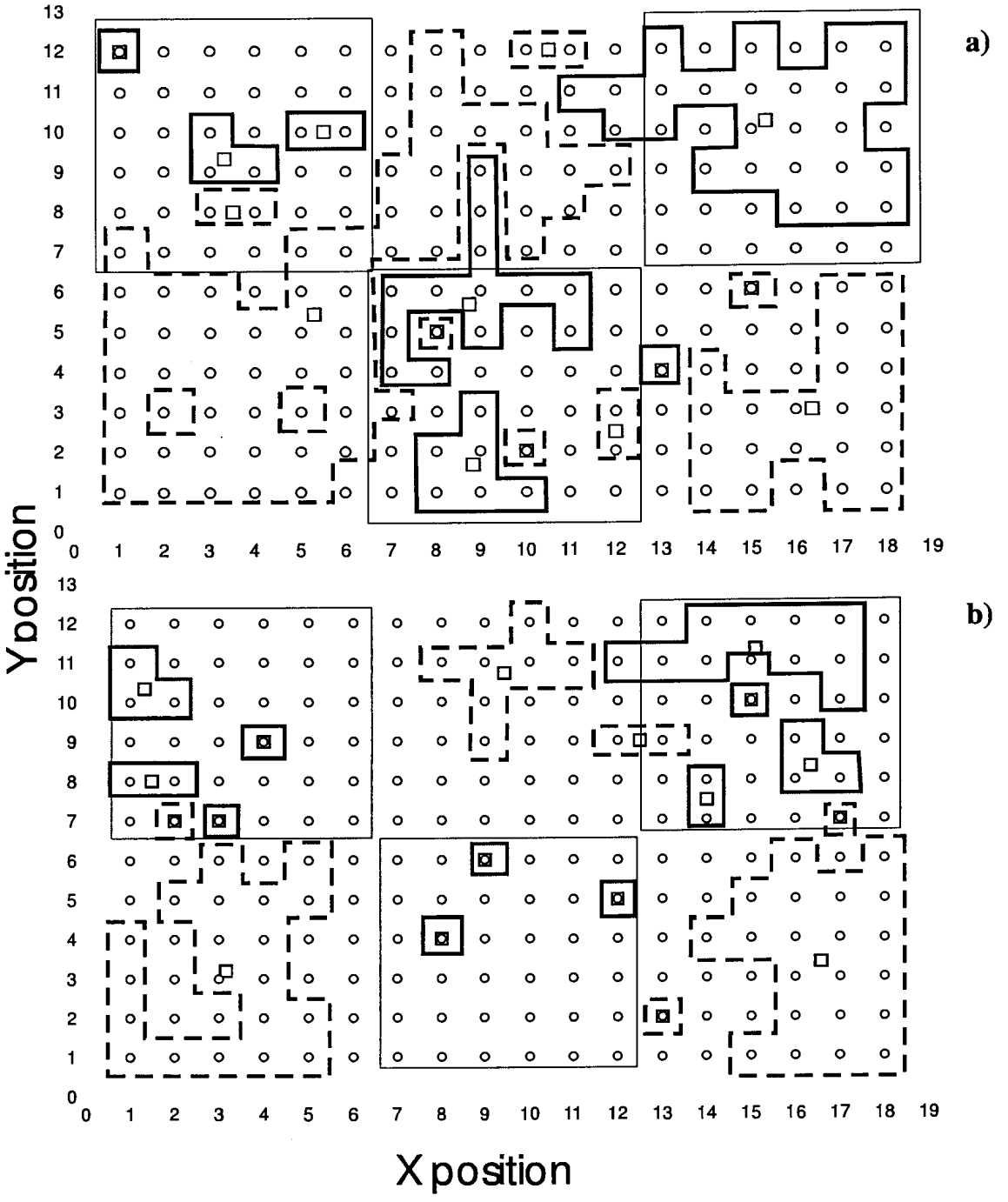

Fig. 5.6. Spatial positions of abundance gaps (dotted outline; $v_j < -1.5$) and patches (thick solid outline, $v_i > 1.5$) for flies with a) W0 (no wing abnormalities) and b) W2 (severe wing abnormalities). Circles represent individual fruit. Squares represent centroids of gaps and patches. Thin solid square outline around groups of circles represent fruit that were shaded by 80 % shade netting (as in Fig. 5.1), with remainder representing fruit exposed to the sun.

The best subset generalised linear model across sampling days, included sampling day, sex, wing abnormality, mean VPD, and the sex by wing abnormality interaction. It explained 43.84 % of the deviance in thorax length, with sex and wing abnormality contributing the most (Table 5.3). Although the thermal environments in the sun and shade plots were different (Table 5.1, Fig. 5.3), there was no effect of treatment (sun or shade) on thorax length in the full model. Rather, next to sex, the extent of abnormality had the largest effect on thorax length, and there was a significant interaction term such that normal and abnormal winged flies differed to a much greater extent in females than in males (Fig. 5.7a). In other words, the pure treatment effect had little significance for thorax size by comparison with exposure to developmentally stressful conditions.

Individual sampling days 9 and 10

For sampling day 9, where abundance of pupated flies was relatively low, the contribution of wing abnormality to the model was small (Table 5.3i). Rather, sex contributed most to the model, although both treatment and abundance, as well as the interaction between treatment and wing abnormality entered the model. However, the contribution of the latter variables was small (see also Fig. 5.7b). In other words, at low abundances, the major effect on thorax length was sex. There were few wing abnormalities at this stage (total = 15), and only a minor effect of treatment (though different for normal and abnormal flies). A different picture emerged for sampling day 10, when abundance was highest (Table 5.3i). Although the contribution of sex was large, wing abnormality explained ten times more variation in thorax length on sampling day 10 than on sampling day 9 (Table 5.3i, total number of abnormally winged flies on day 10 = 177). Fruit mass also contributed significantly to the model on sampling day 10. Indeed, in many ways the outcome for sampling day 10 is similar to that of the whole study, although in this case there was no sex by wing abnormality interaction. In other words, the strongest effects on thorax length are sex and the extent of developmental stress in flies that survive to pupation.

The fact that treatment made little difference to thorax length of flies that survive to pupation suggests that abundance might play an important role in determining size, such that flies in shaded treatments might be experiencing the negative consequences of high densities relative to those in unshaded treatments. However, abundance did not enter the model for sampling day 10 and did so only weakly for sampling day 9 (Table 5.3i). In addition, although cumulative abundance routinely entered the models where it was included in the analyses (Table 5.3ii), it contributed little to the deviance explained. Nonetheless, in this regard it is important to

assess the absolute differences in densities (numbers per fruit) between the different treatments. Figure 5.8 clearly demonstrates that the differences between the two treatments were substantial, and in absolute terms approximately six times as many flies emerged from the shaded compared to the unshaded fruit (6264 vs. 1196). Moreover, the increase in the ratio of abnormal to normal winged flies in the shade vs. sun treatments over days 9 to 11 is striking (Fig. 5.9). A generalised linear model comparing numbers of wing abnormalities whilst controlling for density indicates that this difference is significant and increases through time (Table 5.4). This suggests that the effect of abundance on size might not simply be detectable as an abundance effect in the models, but might also be seen as a change in wing abnormality.

Table 5.3. Best subset generalised linear models for thorax length (in mm) between sampling day, fruit mass, total abundance, treatment (SH = shade), sex (F = female), wing abnormality (ABN = flies with abnormal wings; W0 = no abnormality) and mean vapour pressure deficit (mean VPD) for sampling days 3-11, and for sampling days 9 and 10 separately with either (i) total abundance or (ii) cumulative abundance, the estimate and the estimated percentage deviance explained (% de) by the variables in the model.

Variable	df	Loglikelihood	Chi-Square	Estimate	% de	P <
Sampling day 3-11 (% explained deviance = 43.84, Deviance/df = 0.007, df = 6841)						
Sampling day	1	7231.76	468.53	-0.01	3.98	0.001
Sex	1	7270.37	391.32	0.04 ^{F}	20.47	0.001
Mean VPD	1	7426.30	79.46	-0.04	1.77	0.001
Wing abnormality	1	6623.61	1684.82	-0.08 ^{ABN}	17.62	0.001
Sex x Wing abnormality	1	7442.90	46.26	-0.01		0.001
i. Individual day models with total abundance						
Sampling day 9 (% explained deviance = 38.16, Deviance/df = 0.006, df = 1088)						
Sex	1	1266.16	37.81	0.07 ^{F}	33.11	0.001
Wing abnormality	1	1267.58	34.97	-0.06 ^{ABN}	1.55	0.001
Treatment	1	1281.46	7.21	0.03 ^{SH}	3.13	0.01
Total abundance	1	1281.76	6.60	-0.00	0.37	0.05
Treatment x Wing abnormality	1	1278.12	13.89	0.04		0.001
Sampling day 10 (% explained deviance = 36.65, Deviance/df = 0.007, df = 2330)						
Sex	1	2384.30	161.39	0.04 ^{F}	20.14	0.001
Wing abnormality	1	2205.23	519.52	-0.08 ^{ABN}	15.50	0.001
Fruit mass	1	2446.62	36.75	0.00	1.01	0.001
Sex x Wing abnormality	1	2463.74	2.50	-0.01		0.11
ii. Individual day models with cumulative abundance						
Sampling day 9 (% explained deviance = 38.18, Deviance/df = 0.006, df = 1088)						
Sex	1	1266.39	37.69	0.07 ^{F}	34.00	0.001
Wing abnormality	1	1267.59	35.29	0.06 ^{W0}	1.55	0.001
Treatment	1	1281.46	7.55	0.03 ^{SH}	2.25	0.01
Cumulative abundance	1	1281.76	6.95	-0.00	0.39	0.01
Treatment x Wing abnormality	1	1278.41	13.65	-0.04		0.001
Sampling day 10 (% explained deviance = 36.90, Deviance/df = 0.007, df = 2325)						
Sex	1	2387.98	163.26	0.04 ^{F}	20.14	0.001
Wing abnormality	1	2207.74	523.74	0.08 ^{W0}	15.50	0.001
Fruit mass	1	2451.91	35.40	0.00	0.97	0.001
Cumulative abundance	1	2462.99	9.22	-0.00	0.29	0.01
Sex x Wing abnormality	1	2468.35	2.51	0.01		0.12

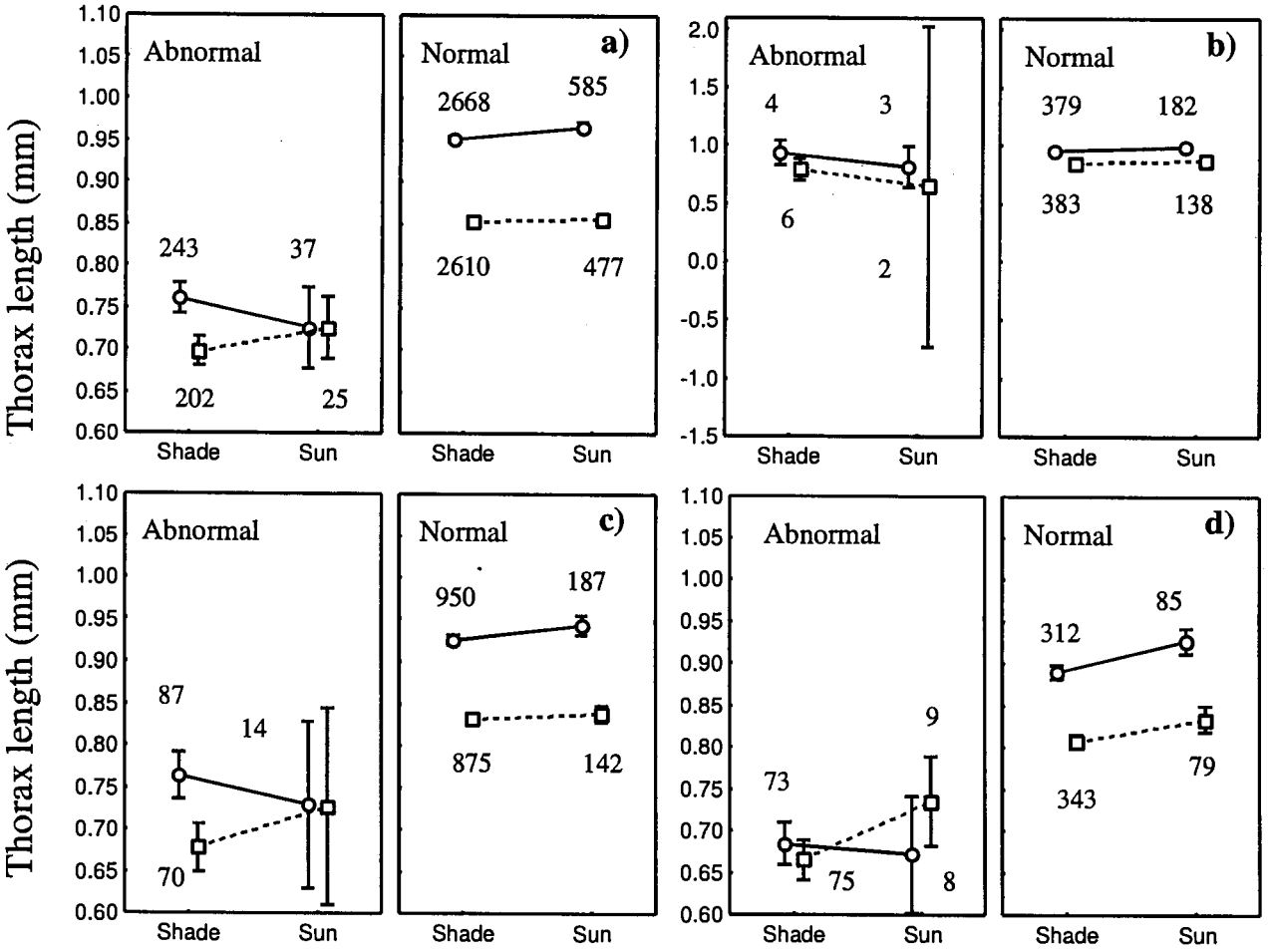

Fig. 5.7. Interaction biplots revealing the differences in thorax lengths (males (squares) and females (circles)) for the abnormally winged (left of each pair) and normally winged (right) flies for a) sampling days 3-11, b) sampling day 9, c) sampling day 10 and d) sampling day 11. Note difference in axis scale for b). Sample sizes inserted above points.

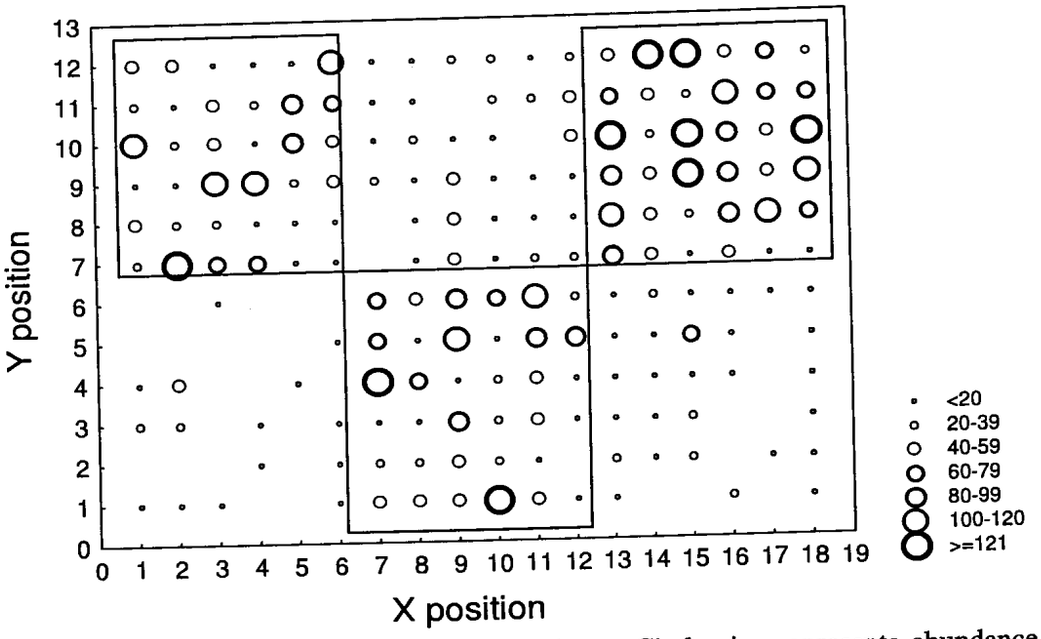

Fig. 5.8. Spatial map of *D. simulans* abundance. Circle size represents abundance of flies in individual fruit. Outlined blocks represent plots that were shaded.

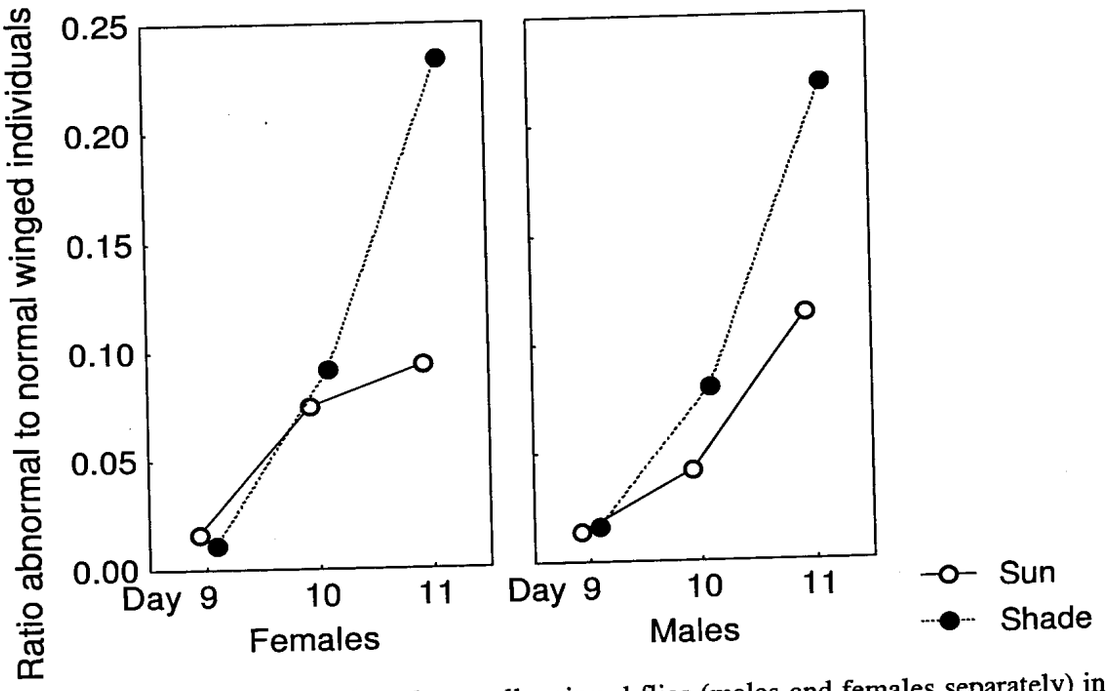

Fig. 5.9. The ratios of normal to abnormally winged flies (males and females separately) in sun and shaded fruit for sampling days 9 through 11.

Table 5.4. Best subset generalised linear model for abundance of flies with abnormally developed wings (for sampling days 9 – 11) between sampling day, total abundance and treatment (S = sun), the estimate and the estimated percentage deviance explained (% de) by the variables in the model.

Variable	df	Loglikelihood	Chi-Square	Estimate	% de	P <
(% explained deviance = 96.82, Deviance/df = 6.81, df = 2)						
Sampling day	2	-91.94	139.37	-8.46	32.71	0.001
Total abundance	2	-62.21	79.92	1.10	18.76	0.001
Treatment	2	-33.57	22.62	-0.50 [S]	45.36	0.001

Discussion

Based on what is known of the responses of *Drosophila* species to temperature, and particularly to stressful high temperatures (reviewed in Hoffmann et al. 2003b), which were realised in this study (Table 5.1, Fig. 5.3), it was predicted that the sun/shade treatment would have a substantial effect on thorax length. By contrast, an interaction between abundance and resource quality would result in little to no spatial structure in thorax length. The lack of any spatial structure in thorax length indicated that there was no simple effect of temperature on thorax length of the emergent adult. That is, the simple developmental effects of temperature differences (Atkinson 1994, David et al. 1997), or the simple effects of stressful temperatures (Feder 1997, Feder et al. 1997a, Feder and Krebs 1998), were overridden by more complex interactions.

It was predicted that this complexity might arise because although thermal stress would be considerable and developmental abnormalities would be common in the unshaded fruit, those larvae capable of finding a refuge from high temperatures in unshaded fruit (Wilmer 1982, Feder 1997) would be capable of realising a large body size. This would be a consequence of a lack of larval crowding and subsequent competition for resources in unshaded fruit, or effects of pre-emptive resource use and subsequent resource pollution (Barker 1983, Scheiring et al. 1984, Hageman et al. 1990, Borash et al. 1998, Sørensen and Loeschcke 2001). This idea was only partially supported. Clearly, the fruit in the sun had much lower final fly abundances than those in the shade (Fig. 5.8), but they also had a lower proportion of abnormally winged flies, especially towards the end of the period (day 11) (Fig. 5.9). This suggests that mortality was high in the unshaded fruit, but that flies in thermal refugia could avoid stress at least to the same extent found in the shaded fruit. From a mechanistic perspective, it is important to determine the

possible reasons both for lower abundances in the sun and higher wing abnormalities in the shade.

The spatial variation in abundance indicated aggregation in the shade treatments, although the treatment effect outweighed simple patterns of aggregation. Other studies of *Drosophila* spp. have revealed aggregation to be a common occurrence (Atkinson and Shorrocks 1984, Wertheim et al. 2002). However, the more important question is what might have led to these treatment effects on abundance. In *Drosophila* spp., aggregated emergence patterns result from a combination of egg clustering, female choice, and patterns of mortality (Atkinson and Shorrocks 1984, Heard and Remer 1997, Remer and Heard 1998, Feder and Krebs 1998, Wertheim et al. 2002). In the field, ovipositing female drosophilids most likely oviposit their eggs in clusters, i.e. lay more than a single egg at a site (Atkinson and Shorrocks 1984, Heard 1998), but avoid fruit if it is warm at the time of oviposition (Feder et al. 1997b). Ovipositing females are unable to distinguish between previously heated and unheated fruit under lower temperature conditions (see Feder et al. 1997b, Feder and Krebs 1998). Therefore, both heat-induced mortality and female choice may play a role in determining emergence patterns. If oviposition takes place early and late in the day, then mortality is likely to be the more significant driver of aggregated patterns of emergences, because females are as likely to lay their eggs in the sun as in the shaded fruit in the cooler periods of the day. By contrast, if oviposition takes place throughout the day, then female choice (i.e. avoidance of hot fruit at the time of egg laying) is likely to be most significant in determining aggregated emergence.

It seems likely that the effect of the sun treatment (and high temperatures) on emergence patterns was mainly via increased mortality, but may also have included a small effect of female choice. *D. simulans* appears to oviposit reasonably constantly over the full day, with very slight peaks at dawn and dusk (David et al. 2004). However, between 10h00 and 15h00, temperatures of the fruit in both treatments exceeded 37 °C, which is presumed to induce mortality in ovipositing females (Feder et al. 2000, Dahlgaard et al. 2001). Thus, it seems likely that the bulk of the oviposition would have taken place during cooler times, when distinction between shaded and unshaded fruit would have been possible only for three hours. For the remainder of the period, ovipositing females are unlikely to have been able to distinguish between previously heated and unheated fruit (see Feder et al. 1997b). Thus, it seems reasonable to assume that mortality was the main driver of low emergence from unshaded fruit. If this was the case, two questions remain to be answered. First, why was the proportion of wing abnormalities initially so similar in the shaded and unshaded fruit? Second, why did these proportions increase so

substantially from sampling day 10 onwards in shaded fruit? Perhaps the most reasonable explanation is that wing abnormalities are unlikely only to be a consequence of thermal stress. Several studies have demonstrated expression of heat shock proteins under high larval densities (Bubli et al. 1998, Sørensen and Loeschcke 2001). If expression is ongoing as a consequence of very high densities then it might be expected that developmental abnormalities would result. Moreover, deterioration of resource conditions as a consequence of high abundances might also mean stressful environments, leading to developmental abnormality. For the case examined here, wing abnormality is likely a consequence of thermal stress in unshaded fruit and crowding in shaded fruit, though direct evidence for this idea is lacking.

Nonetheless, if these explanations are correct, then the initial interactive effects hypothesis is more plausible than one of simple, direct effects of temperature. In shaded fruit there is likely to be substantial competition, resource appropriation and perhaps also resource pollution. Although these conditions do not affect abundances substantially, they do affect size both directly (see also Atkinson 1979) and indirectly. The direct effect is small, e.g. by day 11 (Fig. 5.7d) it is similar to the 7 % difference in size due to crowding reported by Sørensen and Loeschcke (2001) for *D. melanogaster*. Indirect effects via growth and development defects are reflected in the presence of abnormalities and the small size of abnormal flies. By contrast, in unshaded fruit there is high mortality, and some influence on wing abnormality, but normal winged survivors are able to grow to a size equivalent to that of flies in the shade. In other words, the unshaded fruit are a more favourable density environment (see also Feder et al. 1997b) for larvae, whereas the shaded fruit are a more favourable thermal environment. The upshot is little spatial variation in size (Fig. 5.5), but substantial spatial variation in abundance (Fig. 5.8).

This lack of spatial variation in size, but considerable spatial variation in abundance also suggests that the explanation provided by Feder et al. (1997b) for the seeming inability of drosophilids to determine the thermal history of fruit is correct. That is, female flies might specifically avoid high larval density fruit owing to larval movement or might be attracted to low larval density fruit despite their thermal history. Whilst the latter might mean a higher risk of mortality, surviving offspring are likely to stand a lower chance of abnormal wing development and reduced body size than would be the case if they experienced a high density environment. Whether the fitness benefits of laying in a thermally stressful environment would outweigh the costs have yet to be modelled, but this does seem plausible especially in females arriving late at a resource.

The interactive effects hypothesis states that the lethal and non-lethal effects of both temperature and abundance will determine the final outcome of body size. Some support or this hypothesis is gained in this chapter. Additional experiments that reveal that *D. simulans* is equally active in the sun and shade treatments throughout the day and that egg and larval mortality are indeed higher in the sun treatments will give support to the hypothesis of the higher mortality experienced by flies in sun treatments.

Finally, this study has also shown that there is substantial developmental abnormality and mortality in flies developing under natural circumstances. In addition, towards the end of the resource lifespan even normal winged flies can be substantially smaller than those that develop under ideal conditions. Morin et al. (1999) found that under laboratory conditions the thorax length of male and female *D. simulans* from two populations were 0.96 ± 0.003; 0.99 ± 0.004, and 1.08 ± 0.003; 1.09 ± 0.004 mm, respectively, which is much larger than those on sampling day 10 or 11 (Fig. 5.7c, d). Thus, under natural conditions both mortality and non-lethal effects of either temperature or crowding are likely to play a large role in the evolution of body size and need to be given greater empirical attention than has perhaps been the case to date (see Angilletta et al. 2004a, Kozłowski et al. 2004).

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GENERAL CONCLUSION

"…the objectives of the modelling process are to generate questions, test theoretical predictions about the nature of a system, understand causal mechanisms, and integrate the conceptual pieces…" Drake et al. (1996)

Although micro- and mesocosms have been severely criticised for their lack of generality, lack of realism, and short temporal and fine spatial scales (see Jessup et al. 2004, Srivastava et al. 2004), these systems remain useful as conceptual experiments to examine theoretical questions (Drake et al. 1996, Englund and Cooper 2003, Srivastava et al. 2004). The field-based mesocosm used here was particularly suited to examining theoretical questions relating to the effect of a spatially structured environment on the responses of individuals and the realised spatial distributions of species (see General Introduction).

This thesis addressed the following theoretical questions through the use of experimentally derived mesocosm data: i) the influence of the inclusion of natural variation on empirical spatial pattern and its effect on hypothesis generation; ii) the potential for enhancing the hypothesis generating capacity of spatial analytical procedures; iii) the ability of a model (He and Gaston 2000a) to predict the abundances of species from occupancy measures; and iv) the effect of spatial variation in temperature and abundance to determining body size variations under a controlled field situation.

Although natural variation weakened the spatial output, Chapter 2 demonstrated that this did not affect hypothesis generation. Furthermore, hypothesis generation may be enhanced through the use of a sensitivity analysis method developed in Chapter 3. The method partitioned the potential structuring mechanisms into three categories representing extrinsic mechanisms, intrinsic mechanisms and natural variation. Chapter 4 revealed that the He-Gaston model (He and Gaston 2000a) did not predict the abundances of the species inhabiting the nectarines particularly well. Finally, the complex analyses performed in Chapter 5 demonstrated that interactive effects of spatial variation in temperature and larval density were determining the final body size attained by *Drosophila simulans* Sturtevant. Although some progress was made here towards answering spatially related theoretical questions using experimentally derived empirical data, some theoretical and empirical issues remain to be explored.

First, empirical tests of models to predict the abundance of species from their occupancy, or their failure to do so, remain in the minority (for examples see Kunin 1998, He and Gaston

2000a,b, Kunin et al. 2000, Chapter 4). Additional tests of such models are required on a wider range of taxa and across multiple spatial scales to establish the broad utility of these models and the conditions under which they are likely to fail at accurate prediction. The failure of a model to accurately predict abundance may reflect its failure at being sufficiently general to be applicable across taxa, hence reducing its usefulness. This is unlikely to be the case for the He-Gaston model given the previous successes of the model at abundance prediction at local and regional scales using different taxa (He and Gaston 2000a,b, Kunin et al. 2000). Notwithstanding these previous successes, Chapter 4 demonstrated that the model did not perform as well as may be expected when the species under examination were highly aggregated within the finest mapping unit. Consequently, a refinement of the model is required under circumstances of high aggregation within mapping units. As mentioned in Chapter 4, one potential avenue of investigation for refining model estimates might be to include spatially explicit information (see approaches by Perry 1998, Perry et al. 1999). Hartley et al. (2004) have made some progress in attempting to understand the reasons behind the failure of some abundance-prediction models. They found that accurate predictions break down when the mechanisms generating self-similarity (used in their model to predict abundance) change across spatial scales (Hartley et al. 2004). Therefore, an examination of changes in mechanisms with scale and the concomitant changes in abundance and occupancy may also prove to be a fruitful avenue of investigation for the refinement of models predicting abundance.

Second, the findings of laboratory studies may well be incompatible with the situation in the field. In Chapter 5 it was shown that the factors determining body size patterns in the field-collected mesocosm data were not as straightforward as those found under laboratory conditions. Indeed, the interactive and non-lethal effects of temperature and crowding on body size appeared to be just as important as the direct temperature effects that have been established theoretically and in empirical laboratory studies to contribute to body size variations (Chapter 5, Delcour and Lints 1966, Partridge et al. 1994, Roff 2002, Kozłowski et al. 2004). As suggested in Chapter 5, the evolution of body size requires further empirical consideration under field conditions where multiple factors may be interacting in their contribution to body size variation.

On a system-specific level (*sensu* Englund and Cooper 2003), an in depth understanding of the potential mechanisms structuring the *Drosophila* spp. abundance and occupancy patterns examined here was obtained. These mechanisms varied across spatial scales (mapping units). At the finest mapping unit, within fruit, abundance patterns were likely to result from ovipositing females laying their eggs in clutches, and/or, multiple oviposition events in certain fruit (see

Chapters 2, 3). Abundance patterns in slightly larger mapping units, across neighbouring fruit, are likely to be caused by female oviposition behaviour across fruit, i.e. females are more likely to lay eggs in neighbouring fruit (Chapters 2, 3). At much larger mapping unit sizes of 6 x 6 fruit, avoidance of oviposition in sun vs. shade fruit and higher mortality of eggs and larvae in sun fruit are the likely abundance-structuring mechanisms (Chapters 2, 3).

In conclusion, scientific theory needs to be developed hand-in-hand with empirical data gained from both laboratory and field-based studies. Although empirical data collected from field studies at broad spatial and temporal scales are essential for understanding ecosystem level patterns and processes, the collection of such data is difficult. Experimentally derived empirical data from micro- and mesocosm studies remains one means to overcome the difficulties associated with collecting field data while still providing substantial information on pattern and process at multiple scales (see also Englund and Cooper 2003, Simberloff 2004, Srivastava et al. 2004). Indeed, as this thesis has also shown, the potential mechanistic basis of the generated drosophilid abundance and occupancy patterns were identified and shown to vary across mapping units (see Chapter 2, 3). Notwithstanding the system-specific information obtained from this experiment, the mesocosm approach also contributed to the development of theory-based objectives that are applicable at broader spatial scales. For example, this fine scale study has revealed that natural variation is unlikely to obscure the detection of spatial pattern and thus the generation of hypotheses relating to the potential mechanisms structuring biological variables, such as, abundance and species richness. This means that spatial methods, such as autocorrelation functions, are likely to detect pattern at broader scales even with ‘noise’ (natural variation) in the system. The sensitivity analysis approach of Chapter 3 will facilitate in understanding how mechanisms structuring, for example, abundance change with spatial scale and the relative strength of these mechanisms in different taxonomic groups. The use, and development, of spatial analysis (Chapter 2) and the enhanced ability to generate mechanistic hypotheses (Chapter 3) may provide a key to refining abundance-prediction models (Chapter 4, see also Hartley et al. 2004). The use of spatial analysis aided in the understanding of body size variations in the field by facilitating the separation of simple and interactive effects during hypothesis generation (Chapter 5). As a result, while the approach followed is that of traditional ecological research and, at first glance, appears to be “local, experimental and reductionist” (Simberloff 2004), significant theoretical advances, applicable to broader scale issues, may be achieved when using such an approach.

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