Spatial Ecology of the Tansy Beetle (*Chrysolina graminis*)

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For examination for the degree of

Doctor of Philosophy

Department of Biology

University of York

September 2006

Abstract

In Britain, the tansy beetle *Chrysolina graminis* is restricted to the banks of the River Ouse around York where it is monophagous on tansy *Tanacetum vulgare* foliage. After suffering a major decline in England over the last century, effort is now being directed towards the species' conservation. This research aims to inform these conservation measures by investigating the beetles' spatial ecology.

T. vulgare grows in clusters of patches along the Ouse. Beetles are more often found on larger patches that are less isolated from other occupied patches but patch quality apparently plays no role in determining their local distribution. Many regions of bank with seemingly suitable tansy networks were discovered and successful translocations to some of these suggest that this can be an important conservation tool.

Beetle movement behaviour was examined at small scales. No abilities to detect tansy from a distance were evident in either adults or larvae, but both reduce their movement rates when on tansy and speed up when in the inter-patch matrix. Modelling of adult movements yielded accurate predictions of daily movement distances and inter-patch movement rates on local landscape scales and revealed the important role of patch-edge boundaries in restricting movements.

Using multi-patch mark-recapture, dispersal was shown to be female-biased, spatially-localised and directed towards large patches. A dispersal model was developed and fitted to these data, which revealed the significance of the rare long-distance events and showed that beetle dispersal is not seasonal, but is driven by changes in the landscape structure induced by tansy patch growth, flooding and the mowing of patches.

Microsatellite and allozyme markers were developed for *C. graminis*. Using microsatellites, significant genetic structure and isolation-by-distance relationships were uncovered. Populations appeared to diverge when further apart than the beetle's dispersal range and there was evidence that the river is a barrier to dispersal.

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Acknowledgements

I would like to thank my supervisors, Drs. Geoff Oxford and Calvin Dytham, for their advice and help with field, laboratory and computer work over the course of this studentship.

Prof. Jeremy Searle and other members of his research group provided valuable guidance and the use of their laboratory for the microsatellite work, for which I am grateful. Other members of the Biology Department who have given specific assistance include Profs. Richard Law and Mark Williamson.

I would also like to thank Dr. Roger Key (English Nature) for his support of the project and for helping to find funding for the microsatellite work.

Finally, I would like to thank Louise Shields for her encouragement and assistance in collecting beetles.

This research has been funded by NERC, with CASE support from English Nature as part of their Species Recovery Plan and by City of York Council.

Declaration

I declare that the research presented within this thesis is the result of my own work and was written myself with the following exception. The 2003 mark-resight study reported in Chapter 4 was performed by Duncan Sivell.

Chapter 2 was in part published as:

Chapman, D.S., Sivell, D., Oxford, G.S., Dytham, C. & Key, R. (2006) Ecology of the tansy beetle (*Chrysolina graminis*) in Britain. *Naturalist*, **131**, 41-54.

Chapter 4 has been in part submitted for publication in *Oecologia* as:

Chapman, D.S., Dytham, C. & Oxford, G.S. Patterns of landscape-level movement predicted from a model of fine-scale behaviour.

Chapter 5 was in part published as:

Chapman, D.S., Dytham, C. & Oxford, G.S. (2007) Modelling population redistribution in a leaf beetle: an evaluation of alternative dispersal functions. *Journal of Animal Ecology*, **76**, 36-44.

Chapter 1: Thesis introduction

1.1 Introduction

The subject of this thesis is the spatial ecology of the tansy beetle *Chrysolina graminis* L. (Coleoptera: Chrysomelidae) in Britain. Details of the beetle's status and natural history are given in Chapter 2, although it is appropriate to mention here that adult and larval *C. graminis* are specialist herbivores of tansy *Tanacetum vulgare* L. (Asteraceae) foliage. In Britain, the species has declined considerably over the last century, such that it is currently confined to the banks of the River Ouse around York (Oxford *et al.* 2003). Tansy grows in dense stands, which form habitat patches for the beetle. Because these habitat patches are relatively small compared to those in other well-studied systems (e.g. butterfly metapopulations, Hanski 1994, 1999), spatial ecological processes can be investigated in large patch networks, offering a unique opportunity for study. Thus, this work is motivated by a need to understand and conserve the beetle as well as, it is hoped, being of wider interest to spatial ecologists. Some general aims of the research are given below.

1. To monitor and analyse the distribution of *C. graminis* and patterns of *T. vulgare* patch occupancy.

2. To develop an integrated understanding of beetle dispersal, by studying individual movements at varying spatial and temporal scales.

3. To explore spatial patterns of neutral genetic diversity and relate these to dispersal.

4. To make conservation recommendations that will ensure the long-term persistence of the York population of *C. graminis*.

1.2 Background to the thesis – spatial ecology in theory and practice

One of the major advances in ecology over the last half century has been the recognition of the important role of spatial processes (Levin 1974; Kareiva 1990; Kareiva and Wennergren 1995; Lima and Zollner 1996). Spatial heterogeneity is largely ignored by classical ecological models but is an obvious feature of ecological landscapes, for example in the mosaic of man-made habitats that cover much of Britain or changes in vegetation according to clines in climate, topography, soil nutrients and water tables in more natural systems. The spatial dimension of ecological dynamics comes about through the responses of organisms to local conditions (e.g. changes in fitness in different environments) and their ability to move and interact in space.

A thorough review of the entire field of spatial ecology is beyond the scope of this chapter, so here I concentrate on those topics that are most relevant to the thesis. I first discuss the ways in which space is represented in ecological models, with an emphasis on individual-based and patch occupancy models. Since movement and dispersal define the scales at which spatial effects manifest themselves, a more thorough discussion of their consequences and causes then follows. Finally, some demographic and genetic methods of studying movement and dispersal are described.

1.2.1 Representing space in ecological models

Models of spatial ecological processes must represent the spatial distributions of population densities, habitat and resources and also include dispersal and interaction distances. Bolker (2004) categorised such models according to whether they treat space, time and populations as discrete or continuous entities, which I have slightly modified and shown in Table 1.1. In this scheme, three classes of modelling approach are identified – individual-based models (IBMs), lattice models and continuum models. Of these, IBMs and lattice models are of most relevance to this thesis and are discussed below. Special attention is paid to patch occupancy models, as this treatment of space lends itself well to the study of the tansy beetle. Tansy generally grows in dense and discrete clumps along the River Ouse and the beetle's expected reproductive success is greater than zero on tansy and zero elsewhere. Space can thus be represented as networks of discrete tansy clumps, or habitat patches, within a matrix of unprofitable vegetation.

Table 1.1. A modified version of Bolker's (2004) classification of spatial ecological models.

Model	Type*	Space†	Time†	Population†	Stochasticity‡
Cellular automata	I/L	d	d	d	D/S
Coupled map lattices	I/L	d	d	c	D/S
Interacting particle systems (≈ pair approximations)	I/L	d	c	d	S
Integrodifference equations	C	c	d	c	D/S
Spatial point processes (≈ spatial moment equations)	I	c	d/c	d	S
Reaction-diffusion equations	C	c	c	c	D/S
Stochastic patch occupancy models	I	d	d	d	S

* I = individual-based model, L = lattice model, C = continuum model
† d = discrete, c = continuous
‡ D = deterministic, S = stochastic

1.2.1.1 Individual-based models

The prominence of individual-based modelling in ecology has grown massively since the 1980s (Grimm 1999; DeAngelis and Mooij 2005). Although there is no strict definition of an IBM, Grimm and Railsback (2005) set out four criteria that make a model individual-based. First, IBMs model the life history of individuals and the effects of the biotic and abiotic environments on, for example, growth rates and fecundities. Second, IBMs represent resources and their exploitation, rather than use abstract population-level parameters such as carrying capacities or attack rates. Third, population sizes are integers because they are by definition the total number of individuals in the model, whereas in classical models they may be any number. Finally, heterogeneity among individuals is portrayed in IBMs. This can be in terms of the individuals' developmental stage, age, sex, size or location, or may simply be random variation among individual parameter values and behaviour. Such variability is often ignored by classical models, but can have important effects at the population level. For example, individual variation among the diffusion rates in random walk models leads to an increased degree of long-distance movement in the population (Skalski and Gilliam 2003).

IBMs offer the prospect of uniting individual behaviour with population and community-level patterns and processes (Lima and Zollner 1996; DeAngelis and Mooij 2005). This is especially true where the aim of the model is to inquire whether hypothesised behavioural mechanisms underlie particular wider-scale phenomena, i.e. the pattern-oriented modelling approach (Wiegand *et al.* 2003). IBMs have the further advantages of extreme flexibility and the abilities to represent realistic and wide-ranging behaviours and landscapes, heterogeneity among individuals and evolutionary processes (DeAngelis and Mooij 2005; Grimm and Railsback 2005).

However, there are drawbacks to using IBMs. They are often very complex, having been developed for the particular conditions and natural histories of specific populations or communities, and as such may not provide insights into the general properties of ecological systems. Complexity often makes it difficult to fully communicate the model's structure and results (Grimm and Railsback 2005). Furthermore, IBMs are generally mathematically intractable, computationally intensive and data demanding.

A relatively recent advance in approximating spatial IBMs with deterministic mathematics is the development of spatial moment equations (Bolker and Pacala 1997). These are based on reducing spatial point patterns into a set of equations that capture the dynamics and spatial patterns of population densities. The aim is to produce a differential equation describing the first and second moments of the spatial pattern, which are the mean density of each species and the correlations between individual positions of all species respectively and are equivalent to the mean and variance of a non-spatial distribution (Bolker 2004). This method was used by Murrell and Law (2000) to model the movements of carabid beetles in a mosaic landscape of woodland fragments, agricultural land, rivers and urban space.

1.2.1.2 Lattice models

Space is often modelled as a lattice or grid, so lattice models are an important subdivision of IBMs. Lattices are typically composed of square cells (although triangular or hexagonal grids are occasionally used). Spatial heterogeneity can be represented by varying the local conditions inside each cell, summarised as a value or set of values defining its quality or usefulness to the focal organism. With the advent of GIS software, which can combine distribution data with remotely sensed environmental data, it became possible for real landscapes to be represented in lattices (Tischendorf 1997). For this reason, lattice models have been used extensively to solve real-world problems within wildlife and conservation ecology (e.g. Grimm *et al.* 2003; Gardner and Gustafson 2004; Revilla *et al.* 2004). Lattice models are also used to investigate more general questions, such as the evolution of dispersal strategies (Hovestadt *et al.* 2001; Murrell *et al.* 2002) and the effects of habitat loss and fragmentation on extinction risks (Dytham 1994, 1995; Hill and Caswell 1999; With and King 1999). Various neutral landscape algorithms may be used to create artificial habitat quality surfaces over the lattice as the basis for such models (With and King 1997). These include fractal algorithms that are able to produce extremely realistic habitat distributions (Keitt 2000) and can be used to simulate changing landscapes, for example resulting from climate change (Travis and Dytham 2004).

Cellular automata are a class of lattice model that treat each cell as holding one individual, social group or population and generally model cell occupancy or density through discrete time. Interacting particle systems are similar but model continuous time. An advantage of interacting particle systems is the possibility of forming pair approximations to their dynamics (Matsuda *et al.* 1992). These analyse the dynamics of the interacting particle system by concentrating on the joint occupancy probability of neighbouring cells, assuming that pairs of neighbours are independent of one another. For example, Boots and Sasaki (2000) used this method to show that parasites can drive their hosts to extinction if they reduce host fecundity, especially when the parasite has only a small effect on host survival.

Coupled map lattices differ from cellular automata in that they model continuous populations over a lattice landscape (Bolker 2004). The advantage of this approach is that movement can be modelled as continuous space vectors based on distributions of behaviours (e.g. step lengths and turning angles). These can be empirically determined by tracking animals in different habitats (Turchin 1998), meaning that realistic patterns of movement can be represented in the model (e.g. Kindvall 1999). This approach can be applied to cellular automata, but the movement vector must be discretised to the dimensions of the lattice cell, so unless the lattice is of high resolution (small cell size), movement will be poorly represented.

1.2.1.3 Stochastic-patch occupancy models

Stochastic patch occupancy models (SPOMs) are a particularly significant class of spatial IBMs. In a SPOM, the landscape is represented as a network of habitat patches, whereby each patch can be thought of as the individual in the IBM. The occupancies of focal species in each patch are modelled through discrete time. Probably the most influential SPOM is the incidence function model (IFM) of metapopulation dynamics (Hanski 1994, 1999; Ovaskainen and Hanski 2004). To simulate an IFM, one must first calculate the connectivity of each patch, S_i, as,

$$S_i = A_i^{\zeta_{im}} \sum_{j \neq i} A_j^{\zeta_{em}} f(d_{ij}) O_j \qquad \text{Equation 1.1}$$

where A_i is the area of patch i, ζ_{im} and ζ_{em} scale immigration and emigration to A and O_j is the occupancy (1 if occupied and 0 if unoccupied) of patch j at that time. The function $f(d_{ij})$ describes the probability of dispersal over the inter-patch distance d_{ij}. Commonly, the negative exponential function $f = \exp(-\alpha d_{ij})$ is used, where the parameter α is the reciprocal of the mean dispersal distance.

The colonisation probability of unoccupied patches, C_i, is assumed to increase with connectivity as,

$$C_i = \frac{S_i^z}{S_i^z + y} \qquad \text{Equation 1.2}$$

where the parameter z represents the strength of any Allee effect ($z > 1$ gives an effect) and y is a parameter. The extinction probability in an occupied patch, E_i, is related to the patch size, since larger patches will support larger populations and so have lower extinction risks. E_i is calculated as,

$$E_i = (1 - C_i)^r \min\left(\frac{e}{A_i^{\zeta_{ex}}},\ 1\right) \qquad \text{Equation 1.3}$$

where ζ_{ex} scales extinction risk to area and r is the strength of any rescue effect. The metapopulation dynamics of a patch network can be simulated by evaluating E_i and C_i over a number of timesteps and altering occupancies with these probabilities. A deterministic approximation to the SPOM is obtained by replacing the vector of patch occupancies at any one time with a vector of occupancy probabilities averaged over time. This allows the calculation of the metapopulation capacity – the equilibrium proportion of occupied patches – as well as the 'value' of each patch to the metapopulation in terms of its contribution to overall persistence (Ovaskainen and Hanski 2004). In theory, adaptations to the basic model structure can incorporate habitat quality (by modifying patch areas) and matrix elements (by modifying inter-patch distances to account for the resistance to movement in different matrix habitats). The functional forms of the relationships between distance and dispersal, connectivity and colonization or area and extinction may also be varied (Etienne *et al.* 2004; Moilanen 2004).

Two crucial factors behind the success of the IFM are the relative ease with which it can be parameterised from distribution data and the ability to simulate the model using freely available software (Moilanen 2004). The basic data requirements are snapshots of patch networks with patch occupancies by the focal species. If multiple snapshots are available, data on extinctions and colonisations can be used to inform the parameterisation (Etienne *et al.* 2004).

IFMs have been most commonly applied to butterfly metapopulations. For example, Wahlberg *et al.* (2002) parameterised an IFM for the marsh fritillary butterfly *Euphydryas aurinia* and simulated its occupancy dynamics in a landscape of transient habitat patches. Various realistic management scenarios were explored by altering how patches turnover and change in quality through time, with the IFM giving an indication of how such changes may impact on the species. For butterflies, the habitat patches in the model are usually relatively large entities such as meadow fields, or woodland clearings. However, such approaches can also be used at smaller scales, such as for patches of host plants. For example, Biederman (2004, 2005) used a SPOM that was quite similar to the IFM to simulate the dynamics of the leaf beetle *Gonioctena olivacea* on patches of its host plant *Cytisus scoparius*. Host plant patches were subject to high rates of turnover, which meant that high dispersal ability was required for persistence (Biederman 2005). Furthermore, the time to extinction in the model was highly sensitive to the velocity of patch turnover (Biederman 2004).

1.2.2 The role of dispersal in spatial ecology

The dynamics of populations within any local area are the sum of the births, deaths, immigrations and emigrations that occur there, so dispersal is fundamental to population ecology (Clobert *et al.* 2004; Bowler and Benton 2005). Dispersal distance defines the spatial scales at which populations are structured (Thomas and Kunin 1999; Bowler and Benton 2005) and therefore the scales at which space should be studied and modelled. Understanding the causes and consequences of dispersal is a central aim of spatial ecology, particularly as species are forced to respond to shifting habitat distributions due to a warming climate or changing land usage.

1.2.3 Effects of dispersal

1.2.3.1 Effects on individuals

Dispersing often carries a cost to the individual disperser. For example, 99.4% of migrating bird-cherry oat aphids *Rhopalosiphum padi* fail to find suitable hosts in southern Scotland (Ward *et al.* 1998). In species with specialised dispersal morphs such as wing dimorphic insects, the dispersive form may have reduced fecundity and increased age at first reproduction because of extra investment in dispersal (Roff and

Fairbairn 1991). However, while dispersal may decrease fitness for most individuals, a successful disperser may enjoy increased fitness in its new locale as a result of reduced inbreeding and kin competition and/or increased habitat quality (Ebert *et al.* 2002). The dispersal of offspring between patches whose habitat qualities vary unpredictably and independently in time may thus be viewed as a risk-spreading strategy of the parent, since some offspring will survive any local catastrophes (Hanski 2001).

1.2.3.2 Effects on populations and communities

At the level of populations, dispersal has many complex and interacting consequences. Within classical metapopulations, long-term persistence is directly dependent on dispersal, since all subpopulations are eventually destined for extinction (Hanski 1999). However, in small subpopulations, as are common in many insects, dispersal can substantially increase local extinction risk by reducing population size through emigration. This effect may be particularly severe where selection has favoured disproportionately high emigration rates at low densities, such as in the Glanville fritillary butterfly *Melitaea cinxia* (Kuussaari *et al.* 1996). Conversely, positively density-dependent emigration can stabilise the dynamics of subpopulations by reducing excessive densities and smoothing out differences between populations (Aars and Ims 2000). Immigration can also lessen extinction risks by bolstering small populations and reducing inbreeding – a phenomenon known as the rescue effect (Brown and Kodric-Brown 1977). Empirical evidence for the rescue effect is scant but exists circumstantially for *M. cinxia*, where isolation (hence reduced immigration) increases extinction risk in small but not large subpopulations (Hanski 2001). Rescue effects may allow populations to persist in habitat patches that are actually unsuitable for them, provided they are located close to a large population on high quality habitat that provides a continual influx of immigrants, giving rise to source-sink dynamics (Pulliam 1996).

Dispersal has the ability to stabilise and synchronise local dynamics within metapopulations (Kendall *et al.* 2000), although the relationship between dispersal and synchrony is complex and will depend on the dispersal strategy employed (Ranta and Kaitala 2000). Hanski (2001) presents a two-patch model with independent and chaotically fluctuating deterministic population dynamics. After the patches are linked by dispersal, there is a relatively long transient period with increased fluctuations in the dynamics of the metapopulation but no obvious effect on each subpopulation, indicating that the subpopulations have become synchronised. After this transient phase, the two populations settle to out-of-phase two-point limit cycles, which stabilise the metapopulation as a whole. In nature, flying insect populations may be synchronised over long distances (Hanski and Woiwood 1993). At regional scales this is likely to be caused by environmental correlation (for example in weather conditions), while dispersal leads to synchrony at local scales (Sutcliffe *et al.* 1996). Synchrony declines rapidly with distance in less mobile insects, such as the planthopper *Prokelisia crocea*, for whom subpopulations on host-plant patches separated by as little as 10 m are asynchronous (Cronin 2003).

On regional scales, the most important dispersal events are probably the rarest, long-distance ones (Nathan *et al.* 2003). For example, Kot *et al.* (1996) showed that the long-distance dispersers determine invasion speeds, and that 'fat-tailed' dispersal kernels (those with relatively high proportions of long-distance dispersal) may be most realistic. Indeed, the observed spread of the invasive horse chestnut leafminer *Cameraria ohridella* into Germany is only possible if dispersal involved some rare long-distance component (Gilbert *et al.* 2004).

Dispersal also manifests itself at the community level. When species compete for space, evolutionary trade-offs between competitive and dispersive abilities can mitigate this competition and maintain local species richness, provided that habitat is spatially heterogeneous (Mouquet *et al.* 2001). Space, and therefore dispersal, also plays a vital role in natural enemy interactions, such as between predators and prey or parasites and hosts (van Baalen and Hochberg 2001). Huffaker's (1958) pioneering mite experiments provide a classic example of how dispersal permits natural enemies and their victims to coexist when the victim is able to disperse into habitat not occupied by the enemy. Models of spatial predator-prey interactions suggest that such phenomena can generate spatial patterning even in homogeneous landscapes, including travelling waves (Donalson and Nisbet 1999). Waves are known empirically from the dynamics of rabies infections among European red foxes *Vulpes vulpes* (Steck and Wandeler 1980) and in population cycling of the field vole *Microtus agrestis* (Lambin *et al.* 1998).

1.2.3.3 Population genetic consequences

There are profound genetic consequences of dispersal. Low dispersal at the landscape-scale is a pre-requisite for population genetic structure, which will affect the rates of genetic drift and selection and the maintenance of genetic diversity (Whitlock 2001). Island models (those with constant subpopulation sizes and reproductive rates, fixed per capita emigration and immigration rates and a global distribution of dispersal distances) suggest that the effective size of a subdivided population will always exceed that of a panmictic population of equal size (Wright 1939; Whitlock 2001, 2004). The reason for this is that the direction of drift in the different subpopulations is independent so that by chance alleles will achieve high frequencies in some places and low frequencies elsewhere. Thus, a structured population will not lose genetic diversity through drift as quickly as a panmictic one and will have a higher equilibrium genetic diversity.

However, more realistic models that allow for variation in reproductive success, local population sizes and migration rates challenge this assertion. Whitlock and Barton's (1997) formulation suggests that if the variation in reproductive success among subpopulations is less than expected by chance alone (i.e. less than expected from a Poisson distribution), a structured population may retain higher genetic diversity. However, if reproductive success is more variable, the effective population size is reduced, with the results that drift fixes alleles faster and the equilibrium genetic diversity is lower (Wang and Caballero 1999; Whitlock 2004). Since abiotic and biotic conditions vary among habitat patches, it will generally be the case that subdivided populations will vary considerably in reproductive success and should thus have lower genetic diversity than a panmictic population of equivalent size.

The extinction-colonisation process inherent in metapopulation dynamics also acts to reduce effective size, increase the rate of drift and reduce genetic diversity (Wright 1940; Slatkin 1977). This is caused by increased variation in reproductive success.

which will be low in subpopulations on the verge of extinction and high in growing, newly colonised subpopulations (Wang and Caballero 1999; Pannell and Charlesworth 2000; Rousset 2003). If new subpopulations are established by a small number of individuals from genetically similar sources, this will entail extreme founder effects and low-within subpopulation genetic diversity (Pannell and Charlesworth 2000; Whitlock 2001). Conversely, within-subpopulation genetic diversity will be higher if the colonists are many and from a range of distinct subpopulations. Dispersal rates and distances will determine which is the case, although if population turnover has substantially reduced overall genetic diversity to the point where subpopulations are more-or-less identical then dispersal may have little impact on diversity (Pannell and Charlesworth 2000). In expanding populations, the form of the dispersal kernel affects the spatial pattern of genetic variation. If long-distance dispersal is present, the invasion front is characterised by isolated populations ahead of the advancing core, in which founder effects cause long-lasting pockets of distinct genotypes that persist after the expanding core has passed by (Ibrahim *et al.* 1996).

Movement and population structure also affect evolution by natural selection in subdivided populations. For example, high rates of dispersal may hamper local adaptation in heterogeneous habitat patches because of influxes of poorly adapted immigrants (Peck *et al.* 1998; Kisdi 2002), although newly arisen adaptive genes cannot spread from their subpopulation of origin without dispersal (Whitlock 2001). The relatively small subpopulation sizes associated with a structured population will also hinder adaptation as increased drift means that beneficial alleles are less likely to reach fixation (Whitlock 2004). Such processes add an extra component to a population's genetic load (the reduction in mean fitness of a population relative to an optimal genotype), termed the migration load, which results from differing selection coefficients in different habitat patches (Kirkpatrick and Barton 1997). With increasing dispersal rates, migration load is increased but the other forms of load are also altered so that it is not easy to predict how overall genetic load will be affected by dispersal (Whitlock 2004).

1.2.4 Causes of dispersal

1.2.4.1 Ultimate evolutionary causes

Mating with close relatives has negative fitness consequences (Frankham *et al.* 2002) and since natally dispersing individuals will tend to mate with less closely related partners, inbreeding avoidance has been suggested as a cause of dispersal evolution (Bengtsson 1978; Gandon and Michalakis 2001). Sex-biased dispersal may result from this, and is known from vertebrates (e.g. Olson and Shine 2003; Serrano *et al.* 2003; Williams and Rabenold 2005), insects (e.g. Lawrence 1988; Caudill 2003; Conrad *et al.* 2002) and other invertebrates (e.g. King *et al.* 2005). As well as avoiding kin mating, dispersal will also reduce competition between related individuals; so kin selection may also favour dispersal (Hamilton and May 1977; Bach *et al.* 2006). However, the balance between any cooperative and competitive kin interactions and the cost of dispersal will influence the course of dispersal evolution and may select for reduced or increased movement (Bowler and Benton 2005).

Unpredictable environmental stochasticity is also an important process in dispersal evolution (Van Valen 1971; Travis and Dytham 1999), particularly for insects (Roff 1990). When local conditions become harsh, for example through depletion of resources, dispersing may be the only way to survive. Selection for increased dispersal will be strongest when environmental variation is spatially uncorrelated but temporally correlated such that local conditions in the near future are likely to be similar to now but favourable areas may be nearby. Anthropogenic environmental change may also drive dispersal evolution. Habitat loss may select against dispersal (Travis and Dytham 1999), while the exposure to new suitable habitat, for example as a result of climate change or during an invasion, selects for increased dispersal (Travis and Dytham 2002, Simmons and Thomas 2004).

1.2.4.2 Proximate causes

There are many factors that influence individual animals in making the decision of whether or not to disperse. Perhaps the most well known are the dependence of emigration rates on conspecific densities and patch sizes. Dispersal away from areas of high conspecific density will increase fitness by reducing the intensity of exploitative (density effects on *per capita* resources) and interfering (direct density effects through contact with conspecifics) competitive interactions, which can both encourage emigration in insects (Bowler and Benton 2005). Emigration rates may be sensitive to the densities of one particular sex, as is the case for male red milkweed beetles *Tetraopes tetraophthalmus* who disperse away from patches with male-biased sex ratios (Lawrence 1988). Patch size is also likely to influence dispersal rates. Theoretical studies show that emigration will be lower in larger patches because of reduced perimeter-to-area ratios, while immigration will be higher in larger patches, as these are larger targets to dispersers (Byers 1996; Englund and Hambäck 2004). These predictions have been confirmed empirically for many insect species (e.g. Kareiva 1985; Turchin 1986; Hill *et al.* 1996).

Dispersal is also likely to be dependent on the condition of the animal. Indeed dispersers may not be a random subset of the population, but may be those with the most developed dispersive traits or greatest incentive to disperse. For example, male damselflies that dispersed were found to have longer forewings and higher rates of parasitism by water mites than non-dispersers (Conrad *et al.* 2002). Similarly Yang (2000) found that larger stinkbugs *Podisus maculiventris* were more mobile than smaller ones. Hungrier individuals may also increase their movement rates in an attempt to locate food, as is the case for the beetle *Eleodes extricata* (McIntyre and Wiens 1999a), and thus be more likely to disperse. Many insects have specific dispersal stages in their lifecycles, commonly flying prior to reproduction and subsequently losing the ability to fly (Harrison 1980).

Multiple factors may also influence the decision of a disperser to stop and settle in a new patch. Most insects are limited in dispersal ability so will tend to settle close to their source, although there are almost always a small proportion of long-distance dispersers in empirical datasets (Kot *et al.* 1996; Nathan *et al.* 2003). For example, St Pierre and Hendrix (2003) conducted a mark-recapture study on the monophagous weevil *Rhyssomatus lineaticollis* and found that 50% of beetles moved less than 1 m, while the maximum recorded displacement was almost 1 km. Habitat cues used for orientation during dispersal may bias settlement towards patches with high levels of the cue, as is the case for the scarce copper butterfly *Lycaena virgaureae* (Schneider *et al.* 2003). Much may depend on the perceptual range of the species, as if the individual cannot detect better habitat nearby it may settle rather than risk further dispersal.

1.2.5 Methods of studying movement and dispersal

1.2.5.1 Demographic methods

Tracking individuals, for example through radio-telemetry or direct observation, can provide data on the movement behaviour of individual animals in the form of trails or movement paths. Such data can be decomposed into movement vectors consisting of step lengths and turning angles, which can in turn be used to model movement as (correlated) random walks (Turchin 1998). For example, daily movements of the bush cricket *Metrioptera bicolor* were quantified and used to predict dispersal rates between habitat patches over longer time periods (Kindvall 1999). However, it may not always be possible to produce such results because movement is generally not random and individual movement decisions may operate over different scales (Nams 2005). One problem with such models of movement is that heterogeneity in movement behaviour will lead to increased kurtosis in the distribution of movement distances (Skalski and Gilliam 2003) so reproducing long-distance dispersal from mechanistic modelling may be difficult (Nathan *et al.* 2003).

An alternative is to record and analyse data on dispersal events, for example by collecting spatially-referenced individual mark-recapture data. Movement rates between specific patches can be estimated using multi-state mark-recapture models (Hestbeck *et al.* 1991) and potentially analysed as functions of biotic and abiotic variables. However the amount of data needed to estimate inter-patch movement rates for more than a few patches is huge. It is perhaps more feasible to fit a phenomenological dispersal model to the mark-recapture data to infer patterns of dispersal ability. This was the strategy undertaken by Hanski *et al.* (2000) in their development of the Virtual Migration Model (VMM) for inter-patch movement in metapopulations. The VMM is similar in structure to the IFM (see section 1.2.1.3) but takes an individual- rather than a patch-based approach. In the VMM, the probability of emigrating from patch j, ε_j, is a function of the patch area as

$$\varepsilon_j = \eta A_j^{-\zeta_{em}} \qquad \text{Equation 1.4}$$

where η is a parameter. The connectivity of patch j to the network, S_j, is defined as

$$S_j = \sum_{k \neq j} f(d_{jk}) A_k^{\zeta_{im}} \qquad \text{Equation 1.5}$$

where $f = \exp(-\alpha d_{jk})$ is typically used, as in the IFM. The probability of surviving dispersal from patch j, φ_{mj}, rises with increasing connectivity (as dispersal is more likely to be successful) and is given by

$$\varphi_{mj} = \frac{S_j^2}{\lambda + S_j^2} \qquad \text{Equation 1.6}$$

where λ is a further parameter. Surviving individuals are distributed among all target patches in proportion to their contribution to the source patch's connectivity. Making the simplifying assumption that $\zeta_{em} = \zeta_{im}$ and dropping their subscripts, the probability of leaving patch j and reaching patch k, ψ_{jk}, is

$$\psi_{jk} = \frac{f(d_{jk}) A_k^{\zeta}}{\left(\lambda / S_j\right) + S_j} \qquad \text{Equation 1.7}$$

The model parameters can be fitted to multi-site mark-recapture data by maximum likelihood using freely available software (Hanski *et al.* 2000). Applications of the VMM have been to show that dispersal characteristics of butterflies differ between species (Wahlberg *et al.* 2001) and within species in different landscapes (Mennechez *et al.* 2004).

1.2.5.2 Genetic methods

Molecular genetic methods offer the promise of indirect assessments of dispersal, or at least 'effective dispersal' (that resulting in reproduction and gene flow). Particular emphasis has been placed on estimating dispersal from patterns of selectively neutral genetic variation across species' ranges (Gaggiotti 2004). Historically, the most common method was to derive an estimate of effective migration rates from Wright's (1931) F_{ST} statistic, using the following approximation

$$F_{ST} \approx \frac{1}{4Nm+1} \qquad \text{Equation 1.8}$$

where N is the local population size and m is the migration rate. F_{ST} is a measure of the population structure of a single locus and is defined as the probability that two alleles drawn randomly from the same population are identical by descent. As such F_{ST} can be calculated by genotyping individuals from multiple populations. However, this relationship with Nm is based upon many biologically unrealistic assumptions, including no selection, no mutation, constant population sizes and migration rates, random migration, and complete equilibrium (i.e. no spatial structure!) (Whitlock and McCauley 1999).

A class of recently developed assignment methods use multilocus genetic data from multiple populations to try to identify immigrants by assigning each individual to the population its genes are most likely to have originated in (Paetkau *et al.* 1995; Rannala and Mountain 1997; Pritchard *et al.* 2000). In principle, dispersal rates between pairs of populations can then be estimated. However, this requires that all populations are sampled (Beerli 2004) and that dispersal rates are low. Also, some individuals will be assigned as migrants by chance so dispersal rates may be overestimated (Gaggiotti 2004). To address such problems, Wilson and Rannala (2003) developed a multilocus method of analysis to infer migration rates and identify individuals whose recent ancestors were from different populations. This technique is attractive because it relaxes certain restrictive assumptions of earlier models, such as populations being in Hardy-Weinberg equilibrium.

An alternative to directly inferring migration rates, is to simply use the genetic data to characterise the structure of the population, and compare this to demographically acquired knowledge of dispersal or the distances between sampled subpopulations. One way to characterise population structure is to calculate F statistics – F_{ST} (see above), F_{IS} (the probability that two alleles in an individual are identical by descent) and F_{IT} (the inbreeding coefficient for the total population) (Wright 1969). Alternatives include Slatkin's (1995) R_{ST}, an analogue to F_{ST} that is suitable for microsatellite loci (multiple repeats of short non-coding DNA motifs), and the many statistics that describe the genetic distance between populations (e.g. Nei 1987; Goldstein *et al.* 1995).

The differentiation between populations can then be analysed as a function of geographical distance – the 'isolation-by-distance' (IBD) approach. Although the IBD literature contains many methods of assessing the relationship with distance (Nei 1972; Sokal *et al.* 1987; Rosenberg 1989; Slatkin 1993; Costa and Ross 1994), the statistical and evolutionary properties of different genetic distance metrics may be similar so the choice of this may not be critical (Kalinowski 2002). In theory, the strength of IBD relates to dispersal distance, since the rate of increase in differentiation with distance depends on the rate at which gene flow and dispersal decline with distance. Peterson and Denno (1998) calculated IBD for 43 phytophagous insect species and found that moderately dispersive species did exhibit stronger IBD than mobile ones, as expected. However, sedentary species exhibited very weak IBD, which may be because even neighbouring populations of these species are effectively unlinked by dispersal so are as distinct as those further apart.

1.3 Research objectives

The specific goals of the research presented in this thesis are:

- To increase the understanding of *C. graminis*' natural history, especially with regard to oviposition preferences and egg cannibalism.

- To map the distribution of *C. graminis* and its host plant *T. vulgare* along the River Ouse, in order to monitor range fluctuations and analyse the effects of host plant size, isolation and quality and management regimes on beetle occupancy. This will be of general interest as it will test whether spatial ecological theory developed at landscape scales for metapopulations in networks of large habitat patches can be applied to systems with patchy populations occupying small patches relative to the scale of movement.

- To assess the sensory abilities of adult and larval *C. graminis* in terms of their capacities to locate *T. vulgare* from a distance.

- To record and model the movement behaviour of adult *C. graminis* on host plants and in the matrix of non-host vegetation over small timescales.

- To analyse patterns of movement between and within patches over daily timescales in terms of individual and patch characteristics and relate these to movement behaviour through correlated random walk simulations. A major aim of this is to test whether simple models of individual movement behaviour can predict population-level patterns of redistribution, which will be of general interest to modellers seeking to realistically represent movements.

- To develop a general model of inter-patch movement, applicable to *C. graminis* dispersal and show how the model can be parameterised from data on inter-patch movements. The model will be used to evaluate how well several commonly used dispersal kernels (functions describing dispersal ability over different distances) characterise the movement of *C. graminis*. Modellers need to use realistic dispersal kernels and it is hoped that this work will help to inform their choice.

- To develop neutral genetic markers for *C. graminis* and use these to investigate patterns of genetic diversity and population structure across the York population.

Chapter 2: The tansy beetle

2.1 Introduction

The tansy beetle (*Chrysolina graminis* L.) is a large, iridescent green chrysomelid. An observant person walking along the banks of the River Ouse near York in late summer could see hundreds of them feeding on their host plant tansy (*Tanacetum vulgare* L.) and conclude that the species was thriving. However, tansy beetles have almost certainly been lost from all other previously known British sites, leaving them confined to the Yorkshire Ouse. In this chapter, the natural history of the York population is discussed, including the results of some preliminary work that was not taken further in the following chapters. Thus, the aim is to provide a background to the research presented in the rest of this thesis.

2.2 Global distribution

Sivell (2003) compiled information on the global distribution of the tansy beetle, concluding that it is patchily but widely distributed throughout the Palaearctic, with records from most mid, northern and eastern European countries, Russia and Mongolia. Indeed, it is even known from the Taimayr peninsula in northern Siberia (Silfverberg 1994). However, where information has been collected, the species has been found to be rare or declining across its range, with the exception of the Netherlands, where healthy populations apparently still survive (Jaap Winkelman personal communication to Duncan Sivell).

2.3 Distribution in Britain

The distribution of the beetle around York is presented and discussed in detail in Chapter 3. Here the distribution throughout Britain is described. There are 19

British hectads (10 km grid cells) with records of the tansy beetle (Figure 2.1), but it has only been seen in 11 since 1970, six of which are around York. Given that the species is highly visible and spends its days perched on its host plant, it is unlikely to be missed when an area is surveyed, so one can be confident that the eight pre-1970 hectads have lost the species. A problem with determining its former distribution is the high likelihood that many supposed records are actually misidentified *Chrysolina herbacea* (=*menthastri*) (Oxford *et al.* 2003), which occurs on fenland sites in southern England. For example, it is now thought that a record from Redmoor, Cornwall was a misidentified *C. herbacea* based on the presence of the species and the habitat at the site (Geoff Oxford, personal communication) and it seems likely that this is also the case for that from the Scilly Isles. The remaining localities are from fen sites in East Anglia and southern England, although only the populations at Wicken Fen and Woodwalton Fen, both in Cambridgeshire, were definitely *C. graminis*. Despite intensive beetle surveys, the species has not been seen at Wicken Fen since 1981 (Peter Hodge, personal communication to Duncan Sivell), so is likely to have gone locally extinct there. Two visits that were made to Woodwalton fen during this studentship also failed to locate the beetle, so the beetle may have been entirely lost from Cambridgeshire.

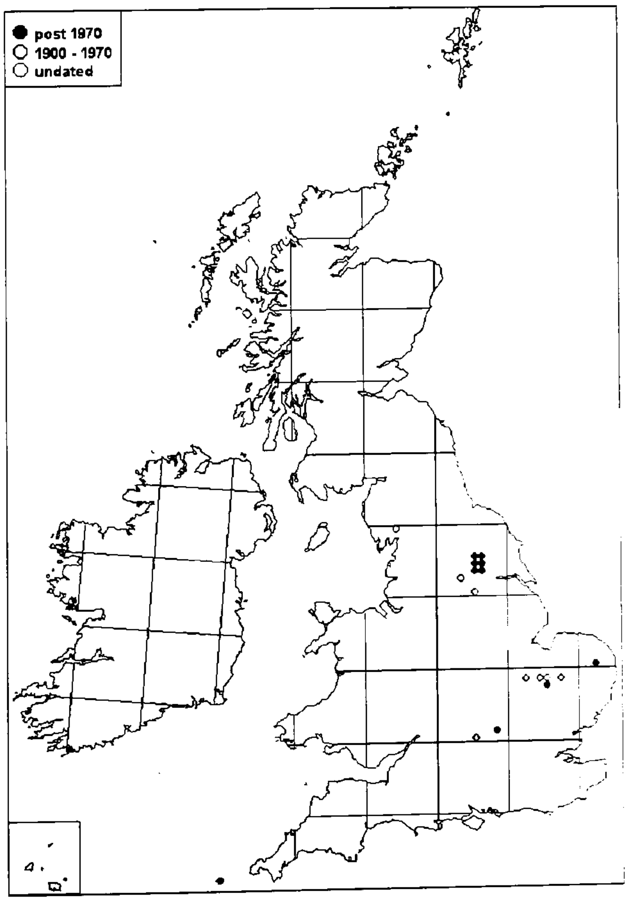


Figure 2.1. A distribution map for the tansy beetle in Britain and Ireland at a 10 km grid cell scale.

The current designation of the tansy beetle in the UK is Nationally Scarce (A) (currently occupying 15-30 hectads) (Hyman and Parson 1992), leading Oxford *et al.* (2003) to remark that the plight of this vulnerable species is woefully understated.

2.4 Host plants

As its name suggests, the principal food plant of both adult and larval *C. graminis* around York is tansy although there are a small number of observations of individuals feeding on other species. At Fulford Ings SSSI (SE6049 and SE6048), adults and larvae have occasionally been seen feeding on gypsywort (*Lycopus europaeus*) and bushy mint (*Mentha x gracilis*) when population densities are high and tansy becomes defoliated. Instances of adults feeding on marsh woundwort (*Stachys palustris*) have also been recorded at Fulford Ings, as well as near Bishopthorpe (SE6047), Wistow Lordship (SE6135) and Nether Poppleton (SE5754). Marsh woundwort is common at other sites such as South Ings SSSI (SE5943 and SE5944) where beetles have never been reported to feed upon it. Beetles feeding on a mint hybrid have also been found at Rawcliffe Meadows (SE5753) (M. Hammond, personal communication to Duncan Sivell).

Although tansy is almost always the plant utilised around York, the population at Wicken Fen fed on water mint (*Mentha aquatica*) and other alternatives reported from abroad include sneezewort (*Achillea ptarmica*), oxeye daisy (*Leucanthemum vulgare*), wormwood (*Artemisia* spp.), hemp nettle (*Galeopsis* spp.) and skullcap (*Scutellaria* spp.) (Sivell 2003). Some of these species grow by the Ouse (e.g. sneezewort and skullcap) yet tansy beetles have never been seen feeding on them. Thus, the York population normally specialises on tansy but sporadically resorts to other species when tansy is unavailable.

2.5 Lifecycle

The beetle's lifecycle is summarised in Figure 2.2. The species is essentially annual, with adults overwintering in the soil. In the spring the majority of individuals emerge from the soil to feed on tansy and mate. Soon after this, females lay their eggs, which hatch into yellow-grey larvae and pass through four developmental instars. Final-instar larvae burrow into the soil and pupate before emerging as new adults in late summer. These feed for a few weeks before returning to the soil to hibernate. Further details of each stage are given below.

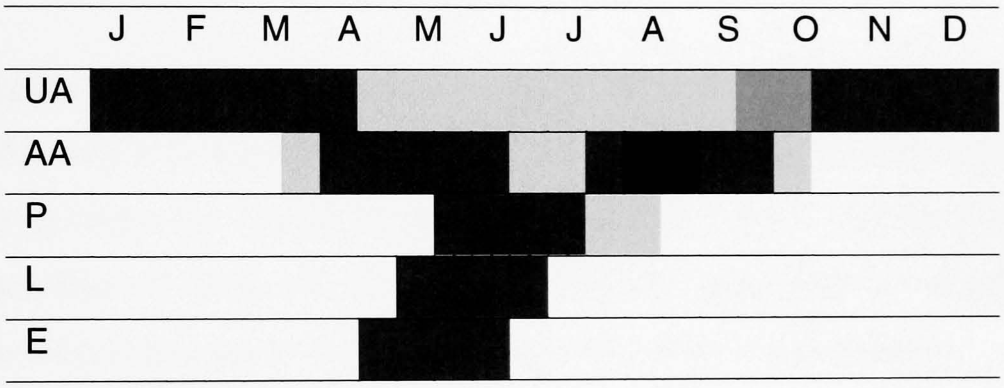


Figure 2.2. A simplified representation of the lifecycle of the tansy beetle around York. The different stages of the beetle are shown in different rows (UA=underground adults, AA=active adults, P=pupae, L=larvae, E=eggs) with darker shades for times when that stage is relatively more common.

2.5.1 Eggs

Tansy beetle eggs are ovoid, around 2 mm long and range from pale yellow to dark orange. They are laid in clusters on the underside of leaves and stems, generally sticking directly out from their substrate. In captivity, one gravid female laid a total of 562 eggs, while another laid 158 (Roma Oxford, personal communication), suggesting that potential fecundity is high. Despite the dependence of larvae on tansy, female beetles often oviposit on non-host species within or around the periphery of tansy patches.

To investigate this, 73 tansy plants occupied by the beetle and a 10 cm zone around these patches were searched for eggs at Fulford Ings SSSI, Middlethorpe Ings

(SE6048) and South Ings SSSI for five minutes per patch in the spring of 2005. Tansy patch basal areas and maximum heights were measured (see Chapter 3) and the numbers of adult beetles on each patch were counted. Sivell (2003) showed that the censused number of adults on a warm, sunny day is approximately half the population size on that patch as estimated with mark-recapture analyses, so relative adult densities were calculated as the census number divided by the patch basal area. Eggs were located on 70 patches, with a mean of 3.63 batches per patch, 61.1% of which were laid off tansy. The mean clutch contained 5.75 eggs (standard deviation of 2.80) and there was no significant difference in the size of clutches laid on or off tansy (*t*-test; N=264, t=0.240, P=0.810).

Laying eggs off the host plant may be a way of avoiding cannibalism of the eggs by other adults, which is regularly observed in the laboratory. However, there was no correlation between the density of adults observed on a patch and the proportion of egg batches laid off tansy (N=70, r=0.102, P=0.402) implying that females do not adapt their oviposition behaviour in response to the risk of cannibalism.

While carrying out these surveys, 40 egg batches (250 eggs) were collected from the field (20 from tansy, 20 from other plants) and the eggs separated from their substrate and each other using paintbrushes and a scalpel with care not to damage them. These were reared individually in tubes with damp cotton wool at 18 °C on a cycle of 16:8 hours of light and dark. Eggs were checked daily for hatching until this occurred or the eggs were clearly decomposing, by which point 205 (82.0%) had hatched. Hatching times were between 4 and 13 days, with a mean of 9.07 days.

An analysis of covariance on hatching time (in days) revealed that eggs laid off tansy (fixed factor, $F_{1,\ 200}$=26.89, P<0.001) in larger clutches (covariate, $F_{1,\ 200}$=49.12, P<0.001) and from larger tansy patches (covariate, $F_{1,\ 200}$=5.703, P=0.018) with low densities of adults (covariate, $F_{1,\ 200}$=5.434, P=0.021) hatched earlier than those that experienced alternative circumstances (Figure 2.3). One interpretation of this is that egg cannibalism by adults was more common at high adult densities and that since both general activity and feeding are concentrated on tansy, eggs laid off the host plant were less likely to be discovered and eaten. Thus, the egg batches collected from tansy in high-density patches hatched later because their increased vulnerability to cannibalism meant they were on average younger.

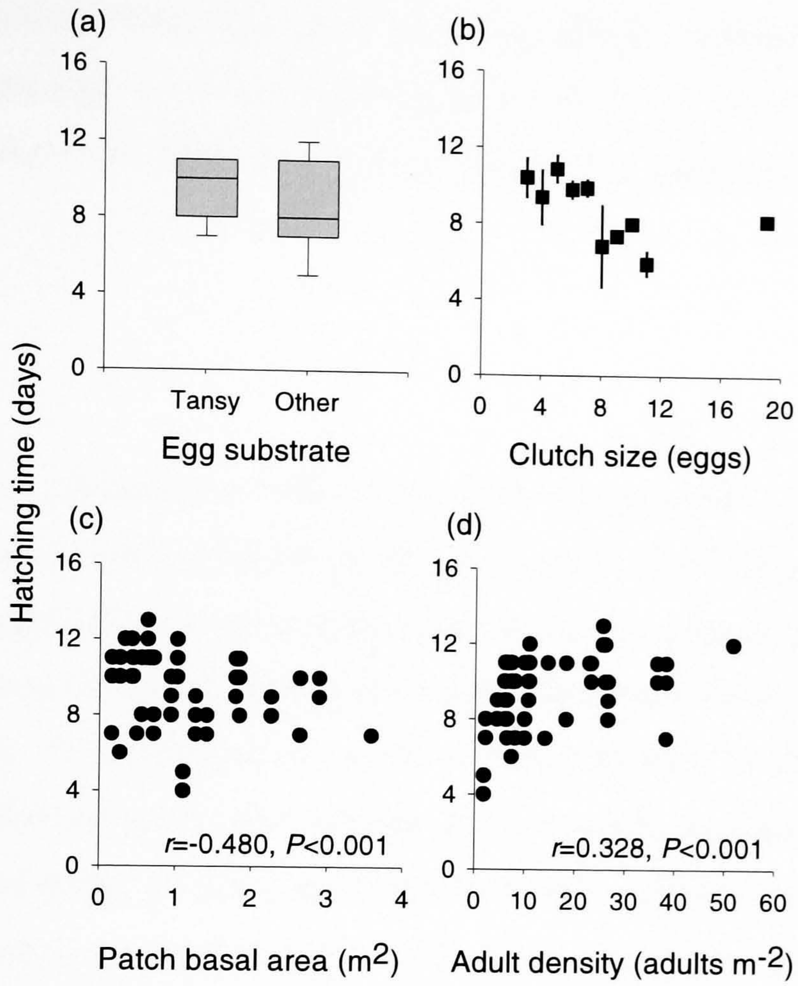


Figure 2.3. (a) Boxplot of hatching times for eggs collected from tansy and other species. Boxes delimit the interquartile ranges with lines through the medians, while whiskers bound the 90th percentiles. (b) Mean hatching times (with 95% confidence intervals) of eggs collected from different sized batches reared singly in the laboratory. Scatter plots of egg hatching time against (c) patch basal area and (d) adult density for eggs collected in those patches and reared singly, with correlation coefficients and *P*-values.

Adults that lay large batches may invest more in their defence, since the clutch represents a higher proportion of total female fecundity. This could account for the finding that eggs from larger batches hatched earlier. Alternatively, higher quality females may lay larger batches of high quality eggs that take less time to develop, which would also result in this. Eggs from large tansy patches might have hatched earlier if large patches created microclimates favourable to egg development or promoted early oviposition. Alternatively, they may have contained more varied oviposition sites that lead to lower rates of egg discovery and cannibalism.

No parasitoids emerged from any reared eggs suggesting that egg parasitism rates must be very low if present at all. This might be due to the presence of toxic defensive compounds. Other *Chrysolina* species both synthesise and sequester defensive chemicals from their host plants including cardenolides that females secrete over their eggs to deter natural enemies (Pasteels *et al.* 1977; Daloze *et al.* 1979; Van Oycke *et al.* 1987; Hilker *et al.* 1992); it is likely that *C. graminis* does the same.

2.5.2 Larvae

The larvae of *C. graminis* are soft-bodied, yellowish-grey and vary in length from around 2 mm when newly hatched up to 1 cm in their final instar. They hatch by bursting their eggshells with specialised spines termed oviruptors (Jolivet and Verma 2002). Since most larvae hatch away from tansy they must move to a tansy plant in order to survive. To investigate the length of time that newly hatched larvae have to do this, 15 larvae (first instar, less than one day old and from eggs reared alone) were deprived of food while 15 others were fed daily with a freshly picked tansy leaflet. Larvae were kept alone in glass tubes with damp cotton wool at 18 °C with 16:8 hours of light to dark. After eight days, none of the fed individuals had died while all the starved larvae were dead, though all survived for at least four days (Figure 2.4). Thus larvae may have a relatively long window of opportunity within which to reach tansy.

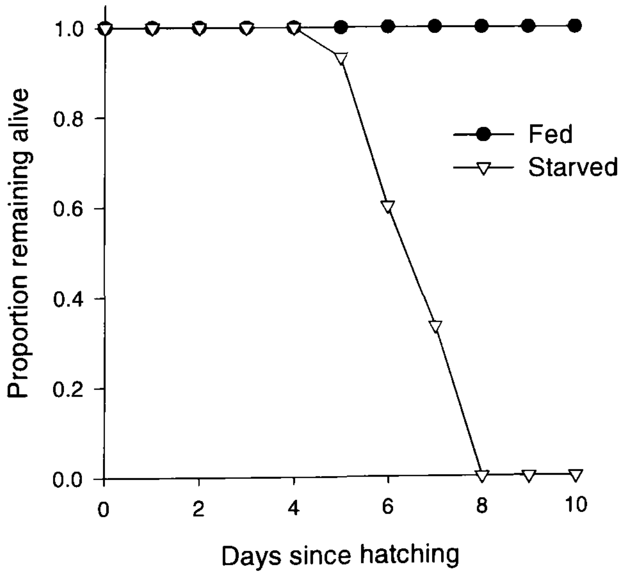


Figure 2.4. Survival through time in the laboratory of two batches of 15 first instar larvae that were either starved since emergence or fed daily with tansy leaves.

One method of supplementing their energy reserves is for newly hatched larvae to eat their own eggshells and cannibalise sibling eggs from the same egg batch (Mafro-Neto and Jolivet 1996), which is observed in the laboratory. To investigate cannibalism, 83 clutches and their substrate leaves were collected from the field and kept in tubes under the same conditions described above. Each day, the batches were checked and all emerging larvae were removed. Data on the fates of individual eggs (hatched or failed) were then collected (eggs were scored as failed once they were clearly decomposed) and collated with those of the eggs reared singly (described above). Logistic regression analysis of egg fates (N=788) showed that those reared with their siblings had a higher chance of failing to hatch than those reared alone (Wald_1=9.543, *P*=0.002) (Figure 2.5) indicating significant levels of egg cannibalism by larvae in their first 24 hours of life. However, clutch size had no significant effect (Wald_1=0.025, *P*=0.875) and larvae from eggs laid on tansy were just as likely to cannibalise their siblings as those from non-host species (Wald_1=0.047, *P*=0.828).

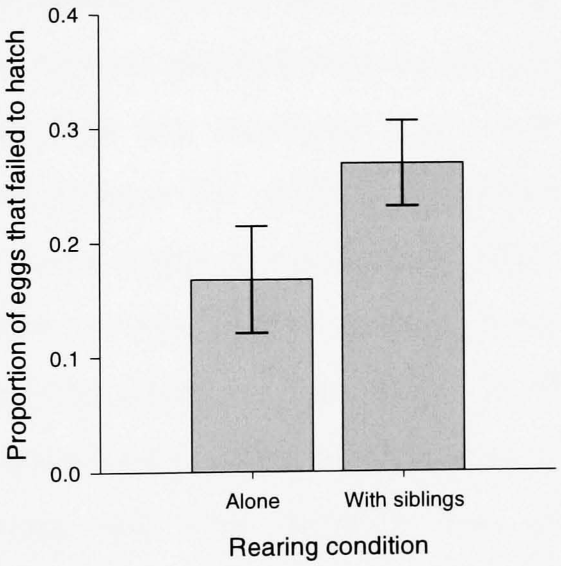


Figure 2.5. Mean egg failure rates (with 95% confidence intervals) when eggs were reared in the lab alone or with their siblings. The higher mortality of those reared with their siblings was due to egg cannibalism by the newly emerged sibling larvae.

Little is known about the survival or dispersal of larvae, although they probably have extremely limited dispersal ability. As preparation for other experiments, 95 field-collected larvae from all developmental instars were reared in the laboratory for up to two weeks (see Chapter 6), during which time no parasitoids emerged. Thus, although a generalist parasitoid tachinid fly *Macquartica tenebricosa* has historically been reported to emerge from *C. graminis* larvae from York (Drummond 1952), these results suggest this is probably not currently a significant source of mortality. Predation is likely to be more important, to which larvae may have some kind of chemical defence similar to that of eggs. A larva being eaten by a scorpionfly *Panorpa germanica* has been observed in the field (Sivell 2003) but other larval predators are not known. However, birds, coccinellids, predatory bugs, lacewing larvae, syrphid larvae, carabids, ants, wasps, spiders and harvestmen all take chrysomelid larvae (Selman 1994) and are common on tansy around York. As a defence against attack, both larvae and adults may occasionally exude reflex blood and, more commonly, exhibit thanatosis (dropping and feigning death), which is an effective escape strategy (Sato *et al.* 2005). From experience of collecting in the field, larvae seem more reluctant to drop than adults, possibly because they are less mobile so have a lower chance of re-locating their host plant and may be of increased vulnerability to ground-foraging predators.

Mowing of the bank can be catastrophic for tansy beetle larvae, probably with death caused both directly by the mowers and indirectly by the loss of food plants. The adult population at Clifton Ings was monitored with mark-recapture techniques through 2004 and 2005 (see Chapter 5). A flood embankment at this site, which holds tansy and beetles, is mown every summer in July while the bank-side patches are left untouched. In 2005, no new adults emerged on any of the mown tansy patches, suggesting that mowing wiped out the larvae. In 2004, these patches did produce adults so mowing must have occurred when at least some larvae had entered the underground pupal stage and were therefore less vulnerable to mowing. Consequently, management for the beetle should be sensitive to annual variation in its phenology. Unseasonal early-summer flooding is also likely to be catastrophic for beetle larvae as they sink and die after a few hours of inundation in the laboratory (Duncan Sivell, personal communication). The summer flooding that occurred in 2000 has been suggested as a cause of the population crashes that were observed in that year (Sivell 2003).

2.5.3 Pupae

Little is known about the pupal biology of the tansy beetle. Final instar larvae burrow a few centimetres down into the soil below their tansy patch before forming leathery yellowish pupae that take 10-11 days to develop into adults. Chrysomelid pupae are exarate (i.e. the appendages remain free) (Jolivet and Verma 2002) meaning that tansy beetle pupae are probably able to respond to disturbance by moving, although any movement is likely to be limited. Possible natural enemies during this stage could include soil-dwelling fungi, nematodes and moles but there are no data on this. Pupae are resistant to flooding, as evidenced by the emergence of new adults after flooding at Clifton Ings in the summer of 2004.

2.5.4 Adults

Adults peak in abundance in August, just after emergence from pupation. Peak abundances vary considerably between years, which in part may reflect the turnover and dynamics of tansy (Sivell 2003). Adult sex ratios have also varied considerably between sites and years, with a mean (weighted by sample size) of 1.096 males per female (Table 2.1). The main activity between emergence and hibernation is feeding, presumably to gain the energy reserves needed to survive the winter, although some mating also occurs at this time. Captive virgin females that mated in autumn but were deprived of access to males in spring were still able to produce viable eggs indicating that they stored sperm over winter (Geoff Oxford, personal communication).

Daily adult survival rates in monitored populations have consistently been estimated at 92 to 96% (Sivell 2003; Chapter 4). Starvation is likely to be a major contributor to mortality when beetles wander away from their host and become lost. However, predation rates are probably relatively low because of thanatosis and defensive chemicals, such as the cardenolides, polyoxygenated steroid glycosides and amino acid derivatives isolated from other chrysomelids (Pasteels *et al.* 1994). Predation rates may be highest during the few days immediately after emergence while the exoskeleton of adults is still hardening. Beetle remains bearing what appeared to be the marks of bird attacks have been found by the river and hardened adults often bear symmetrically indented elytra, as if pinched by a beak while hardening (Sivell 2003). Dead beetles have also been found in the webs of spiders in a captive population. Parasitism may be of lower importance however as no parasitoids have ever emerged from adults kept in the laboratory.

Table 2.1. Sex ratios in adult populations censused as part of different studies. Data from the first seven studies in the table are from Sivell (2003). The Skelton studies of 2003 and 2004 are described in Chapter 4, while details of the Clifton Ings 2004-2005 study can be found in Chapter 5.

Study	Study timing	Beetle number	Males per female
Skelton 2000	Autumn	133	0.985
Skelton 2001	All year	992	0.780
Clifton Ings 2001	All year	201	1.258
Clifton Short Reach 2001	All year	102	1.267
Fulford Ings 2001	All year	643	0.822
Skelton 2002	All year	2096	0.726
Clifton Ings 2002	All year	236	1.126
Skelton 2003	Spring	207	1.876
Skelton 2004	Spring	255	1.236
Clifton Ings 2004-2005	Two years	2226	1.558

Beetles overwinter in the soil below tansy patches and long-term monitoring has indicated that survival during hibernation is surprisingly high, as autumn and spring population sizes are very similar (Sivell 2003). This is despite annual winter flooding of the Ouse, implying that overwintering individuals must be extremely tolerant of long periods of inundation and oxygen deprivation. Beetles must also either choose hibernation sites that are frost-free or be highly resilient to freezing. However, this is perhaps not surprising given that the beetle's range includes Siberia and areas inside the Arctic Circle.

An interesting phenomenon is that around 5% of overwintering adults do not emerge from the soil after the winter, but remain underground for the next year in a state of extended diapause and emerge in the following spring (Sivell 2003). This is probably a risk-spreading strategy allowing populations to recover from the kind of catastrophic effects that can annihilate entire cohorts of active individuals, such as temporary tansy patch destruction by large vertebrate herbivores, and is known from other chrysomelids including the Colorado beetle *Leptinotarsa decemlineata* (Ushantinskaya and Yirkovsky 1976). Many patches suffer annual catastrophes in the form of summer mowing. This kills some of the adult beetles (as the remains of chopped up elytra have been found after mowing) and forces the survivors to move from the mown patch causing them to either starve or reach nearby intact patches (Chapter 5). As the patch re-grows, the diapausing individuals that have persisted through the mowing can emerge and the subpopulation on that patch can recover. Some adults also enter diapause at the end of the breeding period in late May and June, only to re-emerge after the winter for a second active mating season. Diapause is likely to be under hormonal control and may be in response to changes in host plant condition (Jolivet and Verma 2002).

Mating is most common in spring, when copulating pairs can be seen sitting on tansy. In the monitored populations, copulations of the same pair could last over 24 hours, during which time some pairs moved between tansy patches. Mating in a Russian population is preceded by an elaborate ritual involving the male tapping the female's eyes, pronotum and antennae with its antennae (Medvedev and Pavlov 1988). This may be a strategy to prevent inter-specific mating, which is common among *Chrysolina* species in Europe (Jolivet and Verma 2002) though such behaviour has not been observed in the York population, possibly because the extremely similar *C. herbacea* is not present here. Male green dock beetles *Gastrophysa viridula* have twice been recorded attempting to mate with *C. graminis* females around York (Duncan Sivell, personal communication), but the size difference and likely variation in chromosome number between these species probably makes such attempts futile.

As well as being adapted to annual winter flooding, adults also seem able to cope with rare summer floods, which may totally inundate tansy patches. At Clifton Ings, summer flooding does not result in reduced survival as beetles move away from the immediate riverbank and onto the flood embankment. However, some individuals remain stranded on bank-side patches, where they cluster around the top of the plant as floodwaters rise (see photographs in Oxford *et al.* 2003). If the patch becomes submerged, these individuals can be washed away and could then potentially travel long distances downstream. An observed colonisation of the Esplanade (SE5952), downstream of Clifton Ings may have occurred in this manner as the nearest beetle colonies to this site are isolated by scrubby woodland upstream and York City centre downstream, which are likely to be barriers to normal dispersal (Sivell 2003). Stranded beetles have also been seen to crawl down tansy stems into the floodwaters. This could enable them to survive the flood, assuming they can withstand the underwater conditions, perhaps by locating pockets of trapped air in the soil at the base of the plant or by entering a dormant period in which oxygen requirement is reduced.

The principal method of adult dispersal is by walking, as the York population has never been known to fly. Unsurprisingly, their dispersal ability is therefore quite limited. Sivell (2003) marked beetles on one tansy patch and then monitored their dispersal into neighbouring patches over 80 days, finding a maximum net displacement of just over 100 m.

2.6 Conservation

Biodiversity Action Plan (BAP) listing has recently been sought for the tansy beetle, which if secured will heighten its profile as a species requiring conservation action. Since the beetle relies on riverbanks with concentrated tansy patches, measures to ensure the banks of the Ouse are well stocked with tansy should be pursued. Such interventions could include fencing off livestock from parts of the bank and using annual mowing as a method of creating suitable conditions. However, mowing should be responsive to the phenology of the species, with the most appropriate times being in July during pupation and in the autumn when the majority of individuals are below ground. In fact, at South Ings SSSI, English Nature are now carrying out autumnal mowing combined with the avoidance of large tansy patches (Simon Christian, personal communication), which should provide excellent conditions for the beetle.

A simple conservation measure would be to include tansy seed in the mixes sown on newly constructed or repaired flood embankments and other riverside earthworks. Measures should be taken to control invasive plant species on the Ouse, especially Himalayan balsam *Impatiens glandulifera*, which grows in dense stands in many prime beetle locations and in time outcompetes tansy. A further concern is that tansy can be mistaken for ragwort *Senecio jacobaea* by inexperienced or inattentive ragwort-pullers. Since tansy is easily recognised by its distinctive odour, minimal training would prevent this.

The beetle is amenable to captive breeding, as evidenced by populations maintained at the University of York Walled Garden and by Geoff and Roma Oxford (personal communication). Thus, translocations to parts of its former range along the Ouse could be an effective method of increasing the overall range and population size. This would overcome the poor dispersal abilities that are likely to prevent *C. graminis* from naturally colonising suitable areas of bank that are isolated from current populations. One such attempt has been made at between Beningbrough and Linton-on-Ouse and is described in detail in Chapter 3.

2.7 Discussion

The work presented in this chapter has revealed some interesting patterns in the oviposition strategies of *C. graminis*. Most eggs are laid on non-host species, within and around tansy patches. This may reduce the risk of cannibalism by adults, since eggs laid off tansy hatched earlier so may have been on average older than those laid on it. However, females do not lay fewer eggs on tansy when in patches with high adult densities, despite the fact that later hatching of eggs collected from high-density patches points to increased levels of cannibalism there. Even though the movement of larvae may be limited and incur high risks of predation, they have up to 7 days to locate tansy, so may not be at a huge disadvantage if hatching on a non-host species. Evidence of significant larval cannibalism of sibling eggs was uncovered, which will presumably increase this window of opportunity.

No evidence of egg, larval or adult parasitism has been found either in the work presented here or by Sivell (2003), suggesting that few parasites and parasitoids are able to target *C. graminis* at York. This may relate to the species' rarity and range limitation in Britain, and the possible presence of defensive chemicals in its tissues.

The tansy beetle seems adapted to its environment by the employment of simple strategies, such as host-plant specialisation, fixed oviposition preferences and the loss of flight. Unfortunately, this simplicity means it is very vulnerable to change, such as altered water tables and management regimes, which is probably why it has contracted to the York area. However, the prospects for conserving the York population are good, despite the fact that it is still not understand why the beetle thrives on the banks of the River Ouse but not elsewhere in Britain.

Chapter 3: Distribution around York

3.1 Introduction

An understanding of the habitat requirements of species requiring conservation is vital, particularly where their natural histories are poorly known, as is the case for many invertebrates. One approach is to collect spatially-referenced data on habitat variables and the occupancy and/or abundance of the species in order to derive relationships through multiple regression analyses (Augustin *et al.* 1996; Guisan and Zimmerman 2000). However, habitat variables are often collected at spatial scales that are large relative to those over which individuals move and forage, especially for insects (e.g. Fleishman *et al.* 2000; James *et al.* 2003). As a consequence, meaningful statistical relationships with occupancy or density may not be detected.

For herbivorous insects with restricted mobility, the distribution and dynamics of host plants are crucial factors determining site occupancy and population structure (Dennis *et al.* 2003; Biederman 2004). To maintain a viable population, individuals must locate sufficient resources to reproduce, so dense stands of host plants may support higher insect densities per unit of host than sparse plots (the resource concentration hypothesis, Root 1973). Where host plants are patchily distributed, a small cluster of high quality plants in an otherwise unsuitable area may sustain a local population where average site characteristics suggest none should be. Since most habitat studies take little account of variation within a site, this can present a problem in understanding distributions, planning management and identifying 'suitable but empty' habitat patches that are crucial for long-term persistence.

In this chapter, I examine the factors determining the British distribution of the tansy beetle at a scale meaningful to the organism – that of individual patches of its host plant. There is good reason to suspect that *Chrysolina graminis* relies upon dense networks of large tansy plants for its survival at York. The beetle is flightless, and has extremely restricted mobility with higher emigration rates in smaller patches (Sivell 2003; Chapter 5). When occupied, smaller patches are known to hold fewer beetles (Sivell 2003) and are thus expected to be at increased risk of stochastic extinction. It therefore seems likely that larger and less isolated tansy patches would have higher probabilities of occupancy, as is generally predicted by spatial ecological and metapopulation theory (Hanski and Gaggiotti 2004). Additionally, since host plant quality is known to affect the development and survival of leaf beetles (Bernays and Chapman 1994, Obermaier and Zwölfer 1999; Sipura and Tahvanainen 2000; Lower *et al.* 2003), tansy beetles may be restricted to the highest quality tansy patches.

Here, I first review historical patterns in the distribution of the *C. graminis* at York, before presenting the results of surveys mapping the locations of occupied and unoccupied tansy patches along the Ouse to document the current distribution. The spatial structure of the beetle's patch network is analysed within a point pattern framework (Diggle 2003) to test whether or not the distributions of tansy patches and beetle colonies deviate from randomness. The survey data are also used to test the hypothesis that patch size, isolation and quality influence the likelihood of *C. graminis* occupancy. Two complementary approaches are taken – stepwise logistic regression analysis and hierarchical variation partitioning (Chevan and Sutherland 1991; Heikkinen *et al.* 2005) to develop a more complete picture of the factors responsible for the beetles' distribution than can be obtained through any one method alone. Patch occupancies in sections of the banks under different management regimes are then compared to try to identify the management most suitable for the beetle's conservation. Finally, a translocation of beetles to a part of their range from which they have become locally extinct in the last 50 years is described. The spread of beetles from the release sites is documented and the factors affecting the probability of patch colonisation are explored.

3.2 The historical distribution around York

The banks of the River Ouse around York have always been strongholds for the tansy beetle and historical records of the species from Victorian times to the 1990s extend from Linton-on-Ouse (SE4960) in the north to Selby (SE6132) in the south

(Sivell 2003). Sivell (2003) compiled records from the 1990s, sourced from the data of Hammond (1998) and Calvert (1998) at a 1 km square scale. This showed that at this scale, the species had a fairly continuous distribution along the river from north of Nether Poppleton (SE5555) to Riccall Ings (SE6136). Thus, a range contraction about York had apparently occurred in the latter half of the 20th century.

In his own survey of the Ouse, Sivell (2003) considered tansy clumps within five metres of one another to comprise patches of habitat capable of housing independent beetle subpopulations. The positions of these were mapped from Beningbrough (SE5257) to beyond Selby between 2000 and 2003, showing that the beetle occupied just 20-25% of habitat patches. Compared to the records from the 1990s, its range appeared to have shifted south by one to two kilometres but there was no obvious reason for the positions of each range margin as habitat patches continued beyond the last beetle subpopulation.

3.3 The current distribution

3.3.1 Materials and Methods

The current range of the beetle was mapped through surveys at the scale of individual tansy clumps, defined as stems separated by no more than 50 cm and growing in soil (referred to as tansy patches hereafter in this thesis). Some tansy grows in cracks between paving stones in York city centre but these were excluded as they are not habitat for the beetle, which overwinters and pupates in the soil below the patch. In all surveys, patch central coordinates were recorded with a handheld GPS (Garmin 12) and beetle occupancy assessed by inspecting every visible tansy frond for eggs, larvae or adults with minimal physical disturbance. Additionally, other information was recorded to address different questions relating to the range of the beetle (Table 3.1). The specific methods of the different types of survey are described in later sections together with their results. However, all these data were combined to give a picture of the overall range of the beetle around York. Most surveying was performed in 2004, with the exception of the intensive Skelton

(SE5755) survey (summer 2005) and the coarse survey from Wistow Lordship (SE6233) to Selby on the west bank of the river (where access was denied in 2004 because of engineering works). Additionally, the range margins on each bank of the river were located in the summers of 2005 and 2006 to monitor any fluctuations.

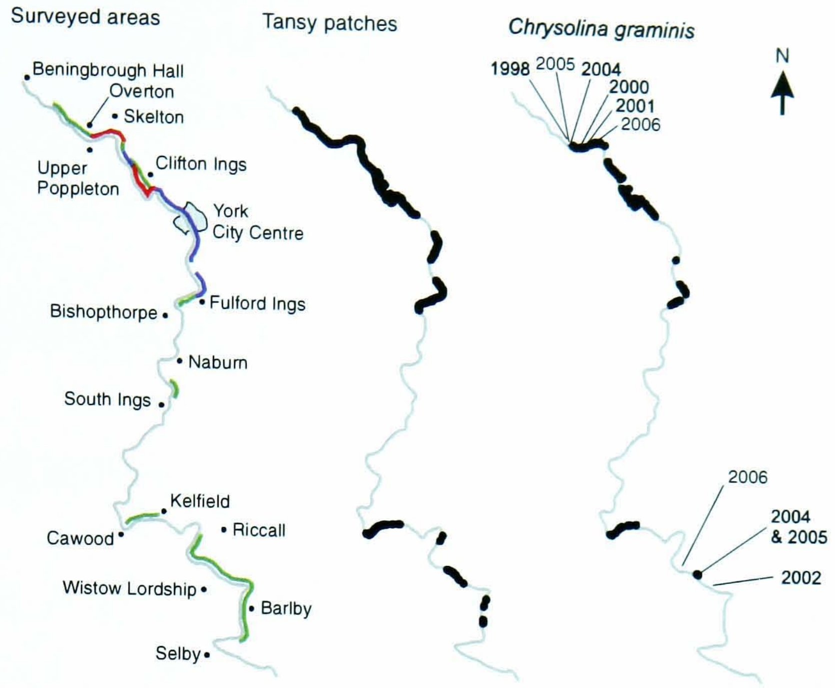
Table 3.1. Types of survey performed to understand and document the current range of the tansy beetle along the River Ouse. Details of the methods of each survey are given in later sections of the main text.

Survey	Timing	Extent	Data recorded other than patch location and beetle occupancy
Coarse	Summer 2004 and 2005	Outside York outer ring-road	Bank-use
Standard	Spring 2004	Within York outer ring-road	Patch size and bank-use
Intensive	Summer 2004 and 2005	Clifton Ings (SE5853) and Skelton (SE5755)	Patch size, data on patch quality and bank-use

3.3.2 Results

The results of these surveys are illustrated in Figure 3.1. Gaps exist where there was no access to the bank but apart from these, the entire British range of the beetle was surveyed, based on previous records around York. Unsurprisingly, the beetle's distribution around York has remained similar to that found in the recent surveys of Sivell (2003). Notable exceptions include increased populations at South Ings and Fulford Ings. Other strong populations were found near Skelton, at Clifton Ings and Middlethorpe Ings (to the north of Bishopthorpe) and also downstream of Nether Poppleton. The precise locations of the range margins on all the banks have fluctuated over a few kilometres but do not appear to be undergoing any sustained expansion or contraction.

(a) East bank



(b) West bank

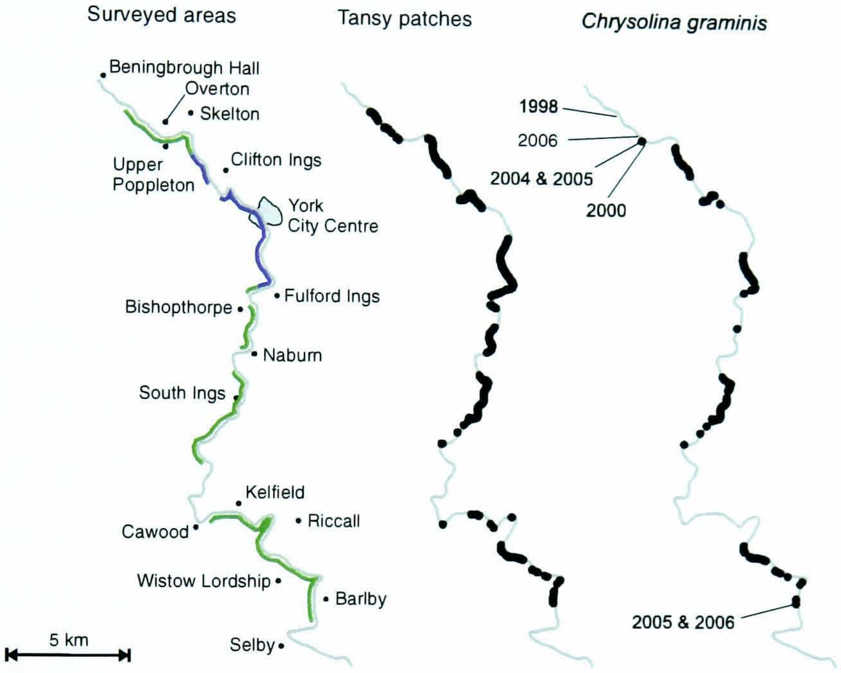


Figure 3.1. The distribution of tansy and tansy beetles around York on (a) the east and (b) the west banks of the River Ouse (grey lines). Surveyed sections of the bank are coloured (green = coarse survey, blue = standard survey and red = intensive survey – see Table 3.1). Records of tansy and *C. graminis* are shown as black dots. All data are from the 2004 surveys, except for downstream of Wistow Lordship on the west bank, which was surveyed in 2005 because there was no access in 2004. The location of the beetle's range margins on each side of the river are labelled in the right-hand maps for years in which it is thought a survey of sufficient scope was carried out. Populations established in translocations (see later in this chapter) are not shown.

Excluding replicated data from the same area (for example at the range margins) 2962 individual tansy patches were found, of which the beetle occupied 38.4%. Although this is higher than the occupancy recorded by Sivell (2003), the figures are not comparable as these surveys were carried out at the level of individual tansy clumps (referred to here as patches) rather than aggregates of neighbouring clumps (which Sivell [2003] referred to as patches).

3.4 Structure of the tansy patch network

3.4.1 Materials and Methods

The standard survey (Table 3.1) gave a snapshot of a large part of the tansy patch network that was used to analyse its spatial structure. In the spring of 2004, tansy patches growing by public rights of way along the Ouse inside York outer ring road were mapped and assessed for occupancy. Additionally, their maximum length, maximum width perpendicular to the length and height of the tallest stem were measured to the nearest 10 cm. Patch basal areas were then estimated as those of ellipses of these lengths and widths.

Maps of surveyed patches are amenable to point pattern analyses (Diggle 2003), which treat the data as a set of unique locations (in this case patches) that may carry some mark (e.g. beetle occupancy or patch size) and are arranged within some region (the east and west riverbanks). The aim of point pattern analysis is to test whether or not the observed distribution of points differs significantly from patterns produced under complete spatial randomness (Diggle 2003). A preliminary step is to define the region that was surveyed for tansy patches. This was not possible in the field so Ordnance Survey maps (1:25000 scale) of the riverbanks were loaded into the GIS program ArcView 3.1 (ESRI) and the surveyed regions on each side of the river were digitised as polygons spanning the footpath to the river's edge.

To analyse the distribution of tansy patches, I modified a basic point pattern statistic – the cumulative frequency distribution of pairwise inter-patch distances or Ripley's K-function (Ripley 1976). To apply this to the dataset consisting of two independent riverbanks, the calculation was stratified among banks, such that the value of K at distance t is given by

$$K(t) = n^{-2} A \sum_{i}^{n} \sum_{j \neq i}^{n} I(d_{ij}, b_i, b_j) \qquad \text{(Equation 3.1)}$$

where n is the number of points in the pattern and A is the area of the surveyed region. The counter-variable I is equal to 1 if both the distance between the centres of patches i and j, d_{ij} is less than or equal to t and the bank upon which patch i is found, b_i, is the same as b_j (east or west) and is otherwise equal to 0. No edge corrections were applied to the calculation of $K(t)$ (see Diggle 2003) because the majority of the edges of the surveyed polygons are 'hard' (Lancaster and Downes 2004), that is representing real boundaries between the riparian belt and the river on one side and terrestrial habitat on the other.

The observed distribution was tested against randomness with a Monte Carlo procedure by simulating 1000 random patterns consisting of the observed number of patches on each bank inside the surveyed regions. The 95% confidence envelope for the K-function under complete spatial randomness was estimated as the 2.5th and 97.5th percentiles of these. At spatial scales for which the observed K-function $K(t)_{\text{obs}}$ lies within the 95% confidence interval of the randomised K-function $K(t)_{\text{csr}}$, the pattern is random, while $K(t)_{\text{obs}} > K(t)_{\text{csr}}$ indicates aggregation and $K(t)_{\text{obs}} < K(t)_{\text{csr}}$ indicates regular spacing. To visualise this across scales, K-functions were linearised as L-functions ($L=\sqrt{[K/\pi]}$) and plotted as the 95% confidence intervals of $L(t)_{\text{obs}} - L(t)_{\text{csr}}$ such that $L(t)_{\text{obs}} - L(t)_{\text{csr}} > 0$ indicates aggregation and $L(t)_{\text{obs}} - L(t)_{\text{csr}} < 0$ indicates regularity (Lancaster and Downes 2006).

A similar method was used to test for spatial autocorrelation in patch areas and beetle occupancy, but this time involving stratified marked point pattern analyses and did not rely on the survey area. Here, a mark-correlation function $K_m(t)$ (Lancaster 2006) was calculated as

$$K_m(t) = \frac{n^{-2} A}{\mu^2} \sum_{i}^{n} \sum_{j \neq i}^{n} I(d_{ij}, b_i, b_j) m_i m_j \qquad \text{(Equation 3.2)}$$

where m_i and m_j are the marks of patches i and j, and μ is the mean value of all marks.

Separate analyses were carried out with the marks being either the patch areas, patch heights or beetle occupancies (0 if unoccupied and 1 if occupied). Significance was again tested by Monte Carlo simulation (1000 randomisations) with random patterns produced by shuffling marks among patches on the same side of the river.

The pattern analysis was conducted using the 'splancs 2.01' package (Rowlinson and Diggle 1993; Bevand and Gebhardt 2000) for the statistical software R (R Development Core Team 2005). The marked point pattern analysis was implemented with a self-coded C++ programme. All analyses were conducted at spatial scales from 5 m to 3000 m (approximately half the maximum inter-patch distance within any one bank) in 5 m steps.

3.4.2 Results

Point pattern analysis showed that tansy patches were significantly aggregated at all spatial scales between 5 and 3000 m (Figure 3.2). Patch areas were negatively autocorrelated at small scales ($t \leq 20$ m), but not significantly different to random at wider scales except for an apparent clustering at moderately wide scales ($2020 \leq t \leq 2695$ m) (Figure 3.3a). This may be a statistical artefact of several regions with similarly sized patches happening to be approximately this distance apart (for example because they lie just beyond the city centre so are regularly mown and kept small). Patch heights were positively autocorrelated at all scales (Figure 3.3b) and beetle colonies were also aggregated over most of this distance ($t \leq 2685$ m) (Figure 3.3c).

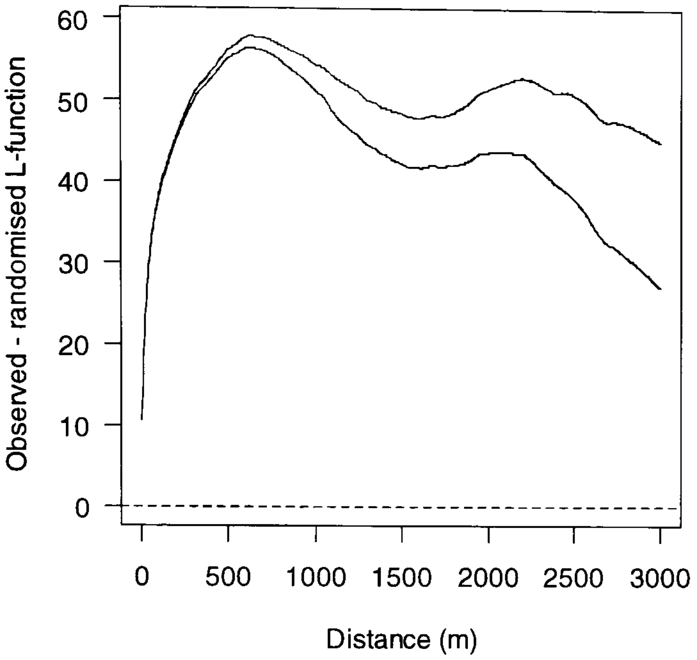


Figure 3.2. Results of point pattern analysis of the tansy patch network within York outer ring road. The L-function is the linearised form of Ripley's K-function, which was calculated for the observed network and for 1000 random patterns. The 95% confidence envelope of the observed L-function minus those of the random patterns is plotted as the are between the solid lines. Since this exceeds zero at all scales, the observed distribution of patches is significantly aggregated.

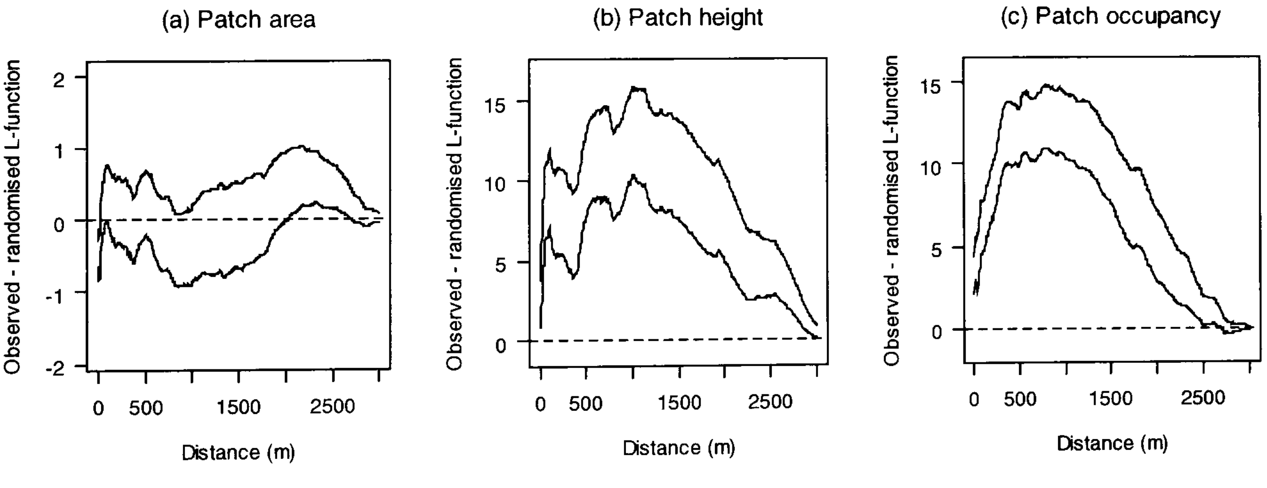


Figure 3.3. Results of marked point pattern analysis on the tansy patch network in the York ring road where the patch marks are (a) patch areas, (b) patch heights and (c) patch occupancies,. The L-functions here are linearised mark correlation functions where the random patterns were produced by randomly shuffling marks between patches on the same side of the river. The 95% confidence envelopes of the observed minus the randomised patterns lie between the solid lines. Where the envelope exceeds zero, the observed marks are significantly positively correlated, while at scales where the envelope is under zero the marks are significantly negatively correlated.

3.5 Effects of patch size, isolation and quality on beetle incidence

3.5.1 Materials and methods

The data from the standard survey (Table 3.1) were used to examine whether or not tansy patch size and connectivity (the inverse of isolation) influenced the likelihood of patch occupancy by *C. graminis*. Data from the intensive survey were used to test the hypothesis that patch quality plays a role in determining occupancy in addition to any effects of size and isolation. These datasets were analysed using the same statistical methods, so are grouped in this section of the chapter. In both the standard and intensive surveys, patch locations, occupancies, basal areas and maximum heights were determined as described above. Below, I first detail the additional methods to collect data on patch quality in the intensive survey, before describing the calculation of patch connectivities and the analysis of patch occupancies for both datasets.

3.5.1.1 Data on patch quality

To determine whether patch quality affects occupancy, intensive surveys were performed just after the emergence of the new generations of adult beetles in August 2004 and 2005 (Table 3.1). As well as patch area, height, locations and occupancy, several possible quality indicators were recorded (Table 3.2). These included the position on the bank, slope and aspect, surrounding vegetation sward height and shading, which may all affect the levels of solar radiation and water received by the patch and thus alter the nutritional value of the foliage. Features of the plants' developmental stages and condition were also recorded (condition of inflorescences, number of fronds and density of groundcover) for similar reasons. Soil hardness was measured since beetles must both overwinter and pupate below ground.

Table 3.2. Tansy patch habitat quality variables measured in the field in addition to patch basal area, height, location and occupancy by *C. graminis* during the intensive surveys (Table 3.1).

Patch quality variable	How measured
Bank slope	Ordinal scale from 0 (flat) to 5 (vertical)
Bank aspect	Recorded with a compass and converted to absolute degrees from south
Number of tansy fronds	Counted
Immature inflorescences	Presence/absence
Mature inflorescences	Presence/absence
Senescent inflorescences	Presence/absence
Density of tansy ground cover in the patch ellipse	Scored to the nearest 10%
Soil unconfined compressive strength	Mean of five penetrometer readings (York Survey Supply Centre EL28-670) taken at random points below the patch (range of 0-4.5 kgfcm^{-1})
Patch position	Riverside (on the steep and cut-away slope leading to the river) or bank-top (above this level on the flatter top)
Surrounding sward height	Mean of the highest stem or leaf measured at five random points immediately surrounding the patch edge
Encroachment and shading by trees and shrubs	Ordinal scale from 0 (none) to 5 (heavily shaded)

In 2004, riverside patches were surveyed along ~2.5 km of Clifton Ings, a prime site for *C. graminis* just north of York city centre. In 2005, ~1.5 km of bank near the northern edge of the species' range near Skelton was surveyed (Figure 3.1a). This region was occupied by beetles in 1998 (Calvert 1998) but underwent a phase of local extinction between then and 2001 such that the range margin lay inside the surveyed area (Sivell 2003). The range then expanded almost up to the 1998 margin by 2005, but subsequently contracted to within the surveyed region by 2006 (Figure 3.1a).

3.5.1.2 Calculation of patch connectivity

Patch connectivities in the sense of Moilanen and Nieminen (2002) were calculated for the standard and intensive datasets, both as the connectivity to the beetle population and the connectivity to the patch network. The connectivity of patch *j* to the population, S_{Pj} is given by,

$$S_{\mathrm{P}j} = \sum_{k \neq j}^{Q} N_k f(d_{jk}) \qquad \text{(Equation 3.3)}$$

where Q is the total number of patches, N_k is the number of adults observed in patch k and $f(d_{jk})$ is a function of the distance between patches k and j, d_{jk} (in metres) that scales the dispersal ability of the species (the dispersal kernel). Thus $S_{\mathrm{P}j}$ is a composite of the distances to other beetles weighted by their ability to cross the distance to j. Based on the results of Chapter 5, a suitable form for the kernel is $1/(1 + 0.310d_{jk}^{1.476})$.

The connectivity of patch j to the tansy patch network $S_{\mathrm{N}j}$ is given by,

$$S_{\mathrm{N}j} = \sum_{k \neq j}^{Q} A_k f(d_{jk}) \qquad \text{(Equation 3.4)}$$

where A_k is the basal area of patch k. Only patches on the same bank and side of York were deemed to be connected, as the river and the city centre are viewed as almost impermeable barriers to dispersal. The connectivities of patches within 150 m of a survey edge were not calculated to minimise edge effects (from the dispersal kernel, patches further apart than this contribute very little to one another's connectivity).

3.5.1.3 Statistical analysis of patch occupancy

The effects of the predictive variables on patch occupancy were tested with logistic regression analyses using binomial generalised linear models (GLMs) as implemented in R (R Development Core Team 2005). If necessary, continuous variables were transformed to improve their conformity to normal distributions. Following Heikkinen *et al.* (2005), ordinal variables (bank slope, shading and density of tansy cover) were treated as continuous. As an initial step, non-linear responses to variables were explored by fitting binomial generalised additive models (GAMs) to the presence/absence data, with all predictors and non-parametric, data-defined smoothers for continuous variables. Quadratic terms were included in subsequent GLM analyses for variables where non-linearity was detected in the smoothed parameters. Backward stepwise logistic regression was then used to produce statistical models of patch occupancy, selecting variables for removal with likelihood ratio tests where $P>0.05$. Once no variables could be removed from the model without a significant change in likelihood, the significance of the inclusion of each excluded variable was tested with likelihood ratio tests.

Three statistics were used to report model fit. These were the proportion of the null deviance explained by the model, the area under the receiver operator curve (AUC), and the point biserial Pearson's product moment correlation between the observed presence/absence and the model-predicted probability of occupancy (Zheng and Agresti 2000). AUC is a threshold-independent, rank-based statistic interpreted as the probability that a randomly chosen occupied patch has a higher model-predicted probability of occupancy than a randomly chosen unoccupied patch (Fielding and Bell 1997).

Multiple correlations among predictive variables (multicollinearity) are commonly found in survey data and present a problem for stepwise regression analyses (Mac Nally 2000; Heikkinen *et al.* 2004). To address this, variation was decomposed among predictors using hierarchical partitioning (Chevan and Sutherland 1991; Heikkinen *et al.* 2004). In hierarchical partitioning, all possible logistic regression models are considered and the average increase in fit (measured by log-likelihood) caused by the inclusion of each predictor across all models is calculated to provide an estimate of its independent and joint effects (Chevan and Sutherland 1991). The result is not a predictive model of occupancy, but rather an indication of the likely causalities and relative importance of each variable. Partitioning was performed within the 'hier.part' R add-on package (Mac Nally and Walsh 2004), including a randomisation routine to test the significance of the independent effects. Since hierarchical partitioning relies on monotonic responses to predictors, the linearity of responses was inspected by smoothing within a binomial GAM and, if necessary, improved with transformations. Because of the large numbers of predictors, only variables selected in the stepwise procedure and others for which a univariate logistic regression based on that variable was significant ($P<0.05$ according to a likelihood ratio test) were included (Heikkinen *et al.* 2004).

3.5.2 Results

3.5.2.1 Patch size and isolation

During the standard survey, 1305 tansy patches were located, of which 348 (26.7%) were occupied by *C. graminis*. Of these, 1206 were selected for the analysis as being >150 m from the survey edges. According to Mann-Whitney U-tests, occupied patches were of greater area (U=198838, P<0.001), taller (U=196422.5, P<0.001) and of higher population connectivity (U=228906.5, P<0.001) than unoccupied ones (Figure 3.4a-c). However, the network connectivities of occupied and unoccupied patches were not statistically distinguishable (U=137844, P=0.978) (Figure 3.4d).

Smoothing within a GAM, suggested that linear parameters for patch area, population connectivity and network connectivity and quadratic parameters for patch height were most appropriate. These were entered into backward stepwise logistic regression, which omitted no terms and confirmed the patterns in Figure 3.4a-c but suggested a negative effect of network connectivity (Table 3.3). From the magnitudes of the Z-scores, population connectivity appeared to be more important than patch area or height. Within the range of measured patch heights (0.1-1.7 m) the quadratic relationship with height was largely asymptotically saturating towards tall patches. The model's fit was good – explaining 44.1% of the null deviance, having an AUC of 0.914 and showing a strong correlation between observed occupancy and its model-predicted probability (Pearson's r=0.679, P<0.001).

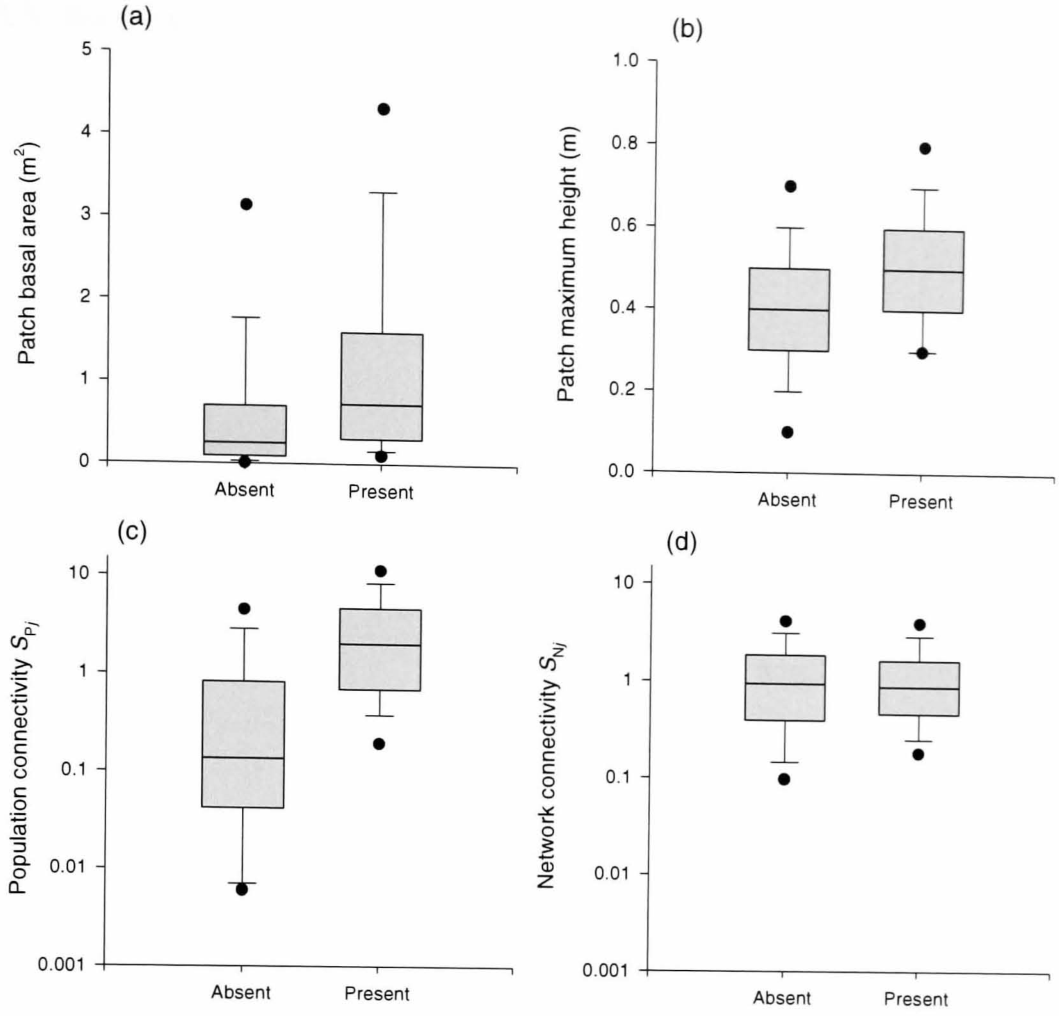


Figure 3.4. Box plots of the (a) basal areas, (b) maximum heights, (c) population connectivities and (d) network connectivities of tansy patches where *C. graminis* was found to be present or absent within the York outer ring road. Boxes delimit the interquartile ranges, with lines through the medians. Whiskers show the 10th and 90th percentiles with dots at the 5th and 95th percentiles. See text for the significance of differences between occupied and unoccupied patches.

All six pairwise combinations of predictive variables were positively correlated at $P<0.001$, with the strongest correlation between patch area and height (Spearman's $r_s=0.541$), indicating collinearity problems in the previous analysis. Since no variables were removed in the stepwise procedure, all were included in hierarchical partitioning, which suggested that the major effects were from population connectivity and area, although all four variables had significant independent effects (Figure 3.5). Some joint effects were negative, indicating that these variables suppress the predictive powers of others (Chevan and Sutherland, 1991). In the case of network connectivity, the suppressive joint effect was of similar magnitude to its independent effect.

Table 3.3. Backward stepwise logistic regression model for beetle occupancy of tansy patches (N=1206) within the standard survey dataset from the York outer ring road, predicted by patch size and connectivity.

Variable	Coefficient (S.E.)	*Z*	*P*
Intercept	-3.108 (0.526)	-5.909	<0.001
Patch basal area (m^2) $^{\ln}$	0.620 (0.083)	7.502	<0.001
Maximum patch height (m)	7.990 (1.577)	5.067	<0.001
Maximum patch height^2 (m^2)	-4.518 (1.158)	-3.902	<0.001
Population connectivity S_{Pj} $^{\ln}$	1.214 (0.087)	14.007	<0.001
Network connectivity S_{Nj} $^{\ln}$	-1.086 (0.123)	-8.844	<0.001

$^{\ln}$ natural log-transformed

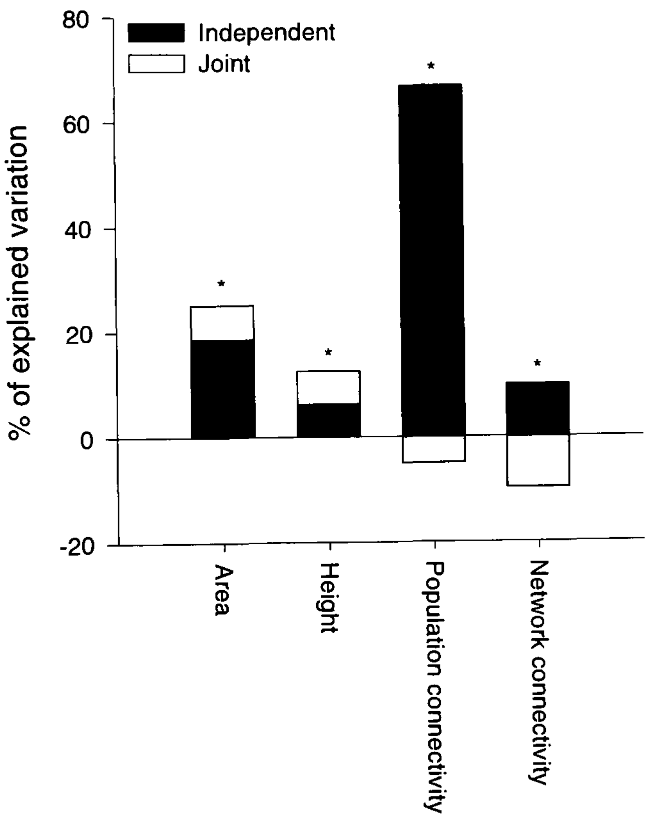


Figure 3.5. Independent and joint effects of (ln) patch basal area, height (squared), (ln) population connectivity and (ln) network connectivity on tansy patch occupancy by *C. graminis*, as estimated by hierarchical partitioning of logistic regressions. Asterisks indicate significant independent effects (P<0.05), according to a randomisation test.

3.5.2.2 Patch quality

During the intensive surveys of patch quality, the number of tansy patches located further than 150 m from the survey limits were 84 for the core area of Clifton Ings (58 occupied) and 49 for the area near the northern range margin at Skelton (29 occupied). These occupancy rates (69.0% and 59.2% respectively) are considerably higher than those found in the standard survey (26.7%), probably because the intensive surveys were carried out shortly after adult emergence and were targeted at areas known to be occupied by the beetle. Smoothing the continuous variables within a GAM suggested that quadratic terms in the logistic regression were only required for sward height. After backward logistic regression, the variables remaining in the model were patch area, population connectivity, density of tansy groundcover and surrounding sward height (Table 3.4). As in the previous analysis, larger and more connected patches were more likely to be occupied. Occupancy was also promoted by increased tansy groundcover. However, the model suggested a humped response to surrounding sward height, with a maximum likelihood of occupancy at ~0.7 m. The model explained 27.5% of the null deviance in occupancy, had a reasonably high AUC score of 0.832 and showed strong correlation between observed occupancy and its model-predicted probability (Pearson's r=0.574, P<0.001).

Table 3.4. Backward stepwise logistic regression model for patch occupancy, using a threshold of P=0.05 in likelihood ratio tests for variable removal. Predictive variables in the initial model related to patch size and connectivity as in Table 3.3, but also patch quality (Table 3.2) and site (Clifton Ings [N=84] or Skelton [N=49]).

Variable	**Coefficient (S.E.)**	***Z* statistic**	***P***
Constant	-1.705 (0.873)	-1.954	0.051
Patch basal area (m^2) $^{\ln}$	0.721 (0.177)	4.077	<0.001
Population connectivity S_{Pj} $^{\ln}$	0.639 (0.164)	3.905	<0.001
Tansy groundcover (%)	0.020 (0.009)	2.162	0.031
Surrounding sward height (m)	4.866 (2.098)	2.320	0.020
Surrounding sward height^2 (m^2)	-3.347 (1.369)	-2.446	0.015

$^{\ln}$ natural log-transformed

There were significant correlations among 45 of the 120 pairs of predictor variables (Spearman's rank correlations with $P<0.05$), with strong correlations between area and height ($r_s=0.540$), area and the number of fronds ($r_s=0.841$), height and sward height ($r=0.566$) and population and network connectivity ($r_s=0.565$). The variables selected in the stepwise regression (area, population connectivity, groundcover and sward height) together with all others giving significant univariate logistic regressions (patch height, the number of fronds and presence of senescent inflorescences) were entered into hierarchical partitioning. To improve the linearity of their responses, patch areas, population connectivities and the numbers of fronds were log-transformed and surrounding sward heights were squared. Population connectivity had the greatest independent effect on occupancy, with the number of tansy fronds and basal area of the patch also having strong independent and joint effects (Figure 3.6). No other variables had significant independent effects, although the presence of senescent inflorescences had a relatively large combined independent and joint effect.

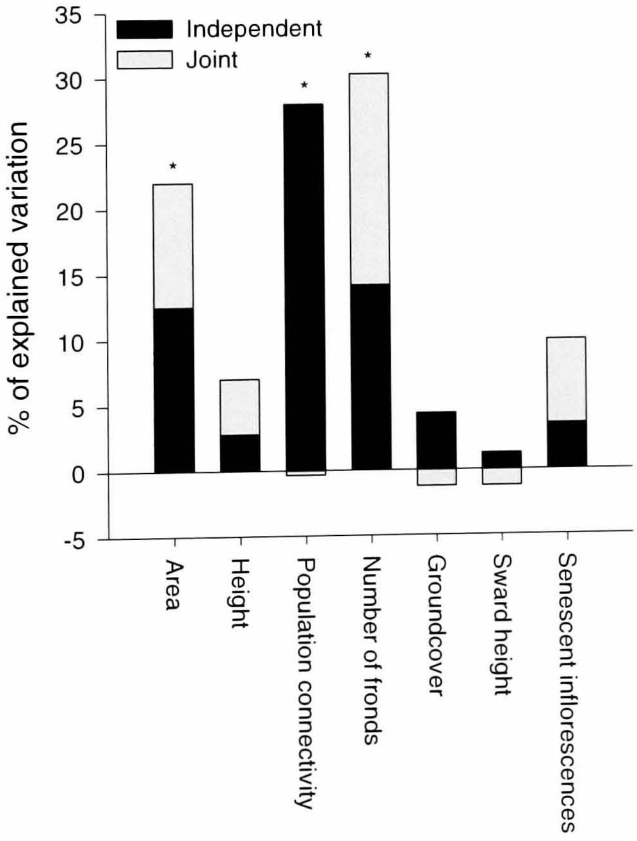


Figure 3.6. Results of hierarchical partitioning of logistic regression models for beetle occupancy on tansy patches, including predictor variables for the effects of patch size, isolation and quality. To improve the linearity of their response, patch area, population connectivity and the number of fronds were log-transformed and sward height was squared. Bars are labelled with asterisks where a randomisation test suggested the independent effect to be significant at $P<0.05$.

3.6 Effects of bank-use on beetle incidence

3.6.1 Materials and Methods

As well as mapping the wider range of *C. graminis*, the coarse survey (Table 3.1) was designed to identify the forms of bank management that create suitable conditions for the beetle. Here, patches were mapped and occupancy assessed but patch sizes were not measured in order to speed up the survey. At each patch the bank-use at that point was classified into one of five categories,

- Unmanaged or mown (since parts of mown banks are always left unmown).
- Cattle grazed.
- Sheep grazed.
- Amenity (principally yacht-moorings).
- Wooded.

Grazing type was determined by the presence of animals, droppings or hoof marks. The majority of the coarse survey was carried out in the late summer of 2004 so only these data were used in the analysis. Because important factors such as patch size and connectivity could not be calculated from these data, their analysis could not be as sophisticated as that presented above. Therefore, a Kruskal-Wallis test (the non-parametric equivalent of one-way ANOVA, Sokal and Rohlf 1995) was used on the presence/absence data (coded 1 and 0 respectively) to test whether or not beetle occupancy was affected by management type, and select the one that best suits the species.

3.6.2 Results

In the coarse survey of 2004, 1641 tansy patches were located and occupancy rates varied significantly among bank-use types (Kruskal-Wallis $\chi^2_4=236.1$, $P<0.001$). Occupancy was higher when the bank was mown or not managed, while amenities, cattle grazing and woodland made banks relatively unsuitable for the beetle (Figure 3.7). Sheep grazed areas were of intermediate suitability, possibly because parts of these are often fenced off to prevent the livestock getting to the river, which could provide a refuge for both tansy and beetles.

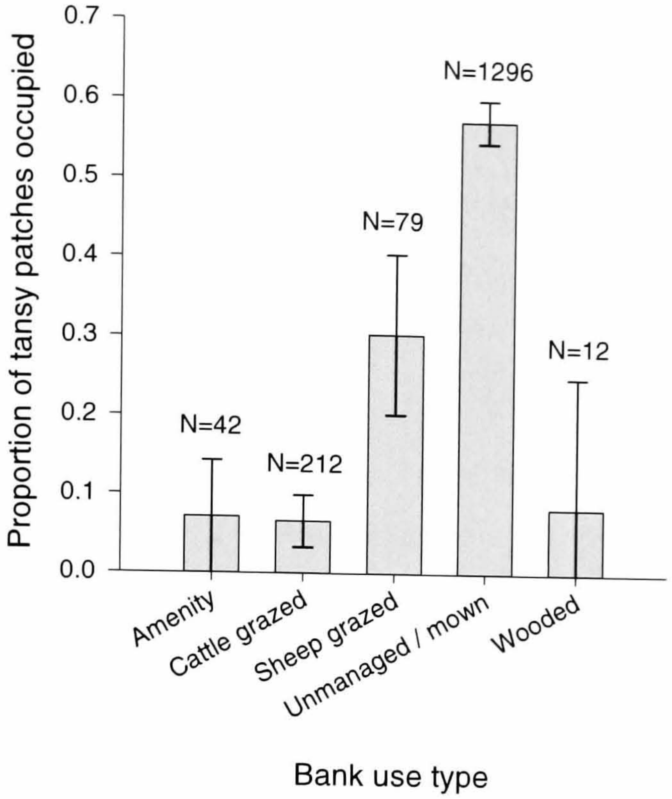


Figure 3.7. Bar chart of the mean patch occupancies (±95% confidence intervals estimated with 1000 bootstraps) of tansy beetles in parts of the bank under different usages. The numbers of tansy patches located in each category are shown above the bars.

3.7 Translocation of the beetle to part of its former range

3.7.1 Materials and methods

3.7.1.1 Translocation and monitoring protocols

Surveys of the beetle's range revealed areas of the bank that were apparently suitable for the beetle in terms of having high densities of large tansy patches but that were not occupied, including places formerly occupied by the species, such as to the north of the current range. Poor dispersal ability combined with fragmentation of the bank through inappropriate management may have prevented it from reaching these areas after local extinctions. Since the beetle is easy to collect from the field and rear in captivity, translocation may be an efficient management tool for the species' conservation.

Towards this end, five translocations were carried out in the spring of 2005 to apparently suitable bank between Beningbrough Hall and Linton-on-Ouse, approximately 5 km upstream of the current natural range, (Figure 3.8). Historically the beetle was present in this area (Mary Thallon, personal communication to Geoff Oxford), but was not recorded there during the 1990s (Calvert 1998; Hammond 1998), by Sivell (2003) or when the region was visited in 2004. Between Beningbrough Hall and the recent range margins, there are a number of potential barriers to dispersal such as densely wooded, overgrown and grazed bank that may prevent natural recolonisation. Thus, if the former population went extinct stochastically or as a result of a temporary catastrophe, such as unseasonal flooding or a period of overgrazing, from which the habitat has recovered, it is unlikely that recolonisation could occur in the near future.

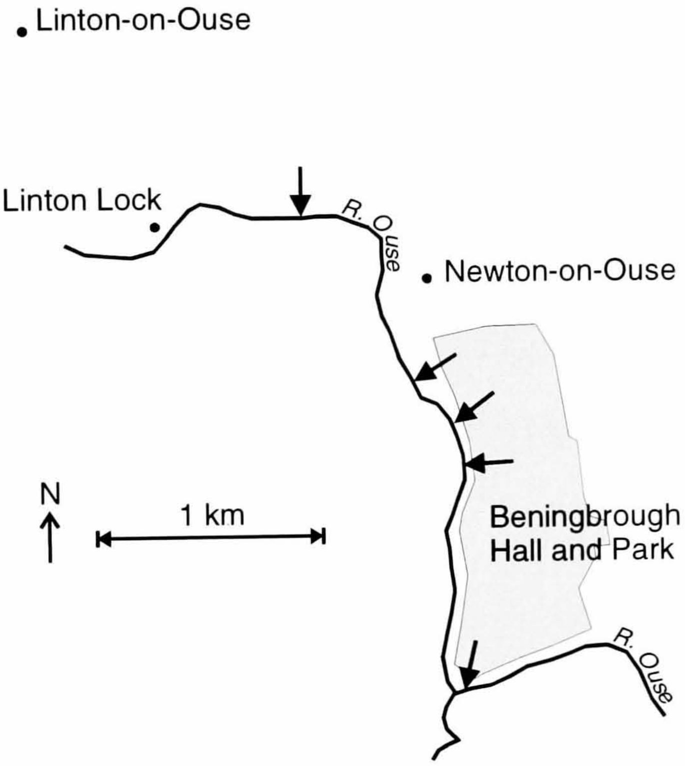


Figure 3.8. The locations of five releases made at Beningbrough Hall and Newton-on-Ouse (arrows pointing to the River Ouse) as part of the translocation programme.

Beetles were collected from the strong population at South Ings and also from a captive population held at the University of York (originally derived from material collected throughout the beetle's range). During each of the five releases, 30 beetles were placed onto two or three neighbouring tansy patches on 13th May 2005. Release locations were selected on the basis of being large tansy patches with closely situated neighbours. The sizes of all the release patches were measured and their coordinates were recorded as in the standard survey (Table 3.1). To assess the extent to which the beetles had spread and their population size, the areas were revisited on 9th August 2005, 8th June 2006 and 30th August 2006 and surveyed following the standard survey procedures, but also counting the number of beetles found.

3.7.1.2 Analysis of survey data

Logistic regression analysis was used to assess the importance of tansy patch size and connectivity on colonisation by the beetle. Population connectivities were calculated from the survey data as in Equation 3.3, but replacing the term for the number of beetles with patch occupancy (0 = unoccupied, 1 = occupied). The reason for this is that beetle population sizes are much smaller in spring than summer since the new generations emerge from pupation in late summer so population connectivities based on the number of beetles censused in the spring surveys would be artificially low compared to those in summer. Sivell (2003) found that in good weather, roughly half the beetles on a patch are seen so patches with more than a few beetles on them are likely to be recorded as occupied. Thus, using occupancies instead of population sizes somewhat reduces this bias. Since the survey data went beyond the local ranges of the beetle, edge effects did not require consideration unlike in previous analyses.

For each set of survey data, current and historical population connectivities to patches within each site (Beningbrough Hall or Newton-on-Ouse) were calculated, where historical connectivities were those to occupied patches in the previous survey data. To ensure that the analysis was only carried out on newly colonised tansy patches, those within 10 m of an occupied patch recorded in the previous survey were excluded. A threshold of 10 m was chosen to conservatively account for GPS error, which is generally in the region of 3-5 m. With these data, backwards stepwise logistic regression and hierarchical variation partitioning were used to assess the effects of tansy patch area, height, current connectivity, historical connectivity and site on colonisation (0 = not colonised, 1 = colonised), following the same methods as those in section 3.5.1.3.

3.7.2 Results

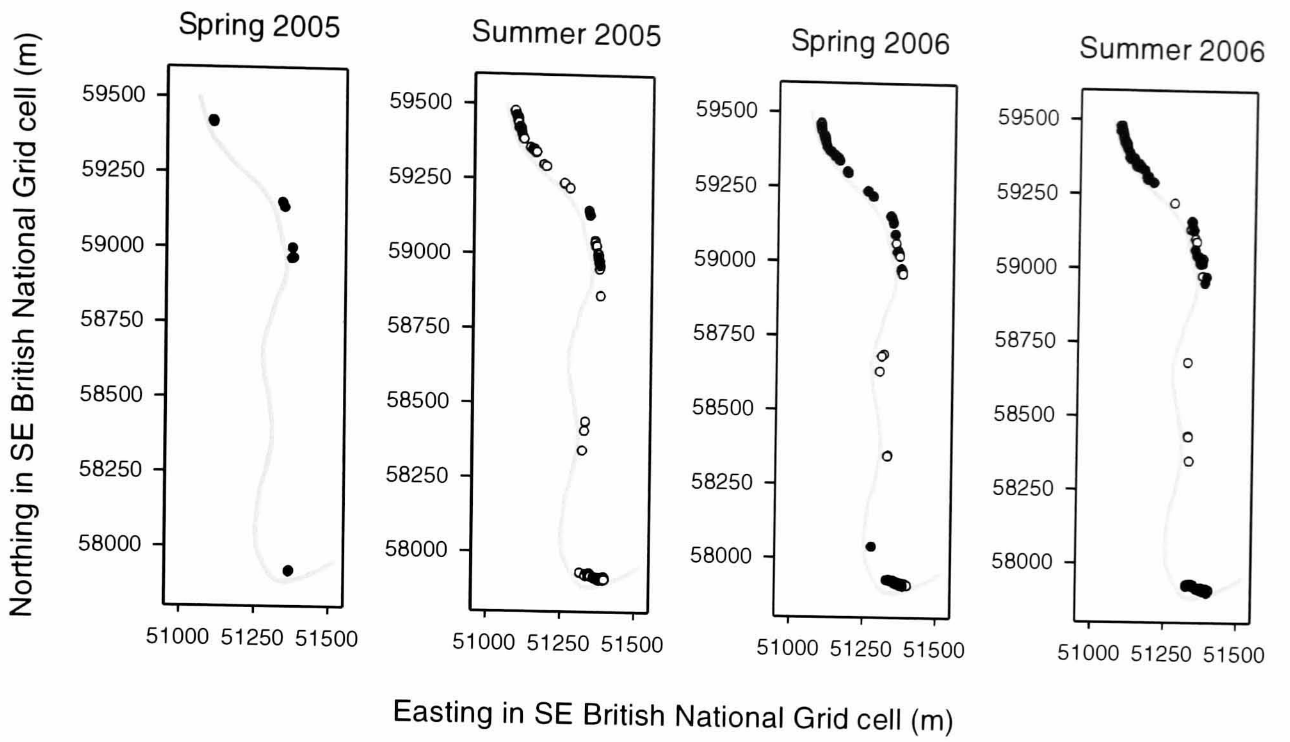
3.7.2.1 Spread of beetles following release

Figure 3.9 shows the expansion of beetles after translocation. Beetles were found close to where all five releases were made during each survey, indicating that breeding populations were established after all translocations. Beetles mostly spread onto nearby patches, which would be expected if their dispersal ability were limited. The expansion was evident in the recorded patch occupancy rates, which continued to increase rapidly (Table 3.5). However, the numbers of beetles counted in the summer surveys (when population sizes peak after emergence from pupation) suggested the population was not growing as rapidly as its range, indeed an apparent decline occurred between 2005 and 2006.

3.7.2.2 Factors affecting patch colonisation

After patches within 10 m of previously recorded occupied patches were removed from the survey data, 220 remained, of which 52 were colonised during the monitoring. Colonised patches were of greater areas (Mann-Whitney U-test, U=-2.922, P=0.003), taller (U=-2.745, P=0.006) and better connected to both the current beetle population (U=-5.313, P<0.001) and that recorded previously (U=-2.625, P=0.009) than those that were not colonised. Smoothing within a binomial GAM suggested that linear parameters were appropriate in logistic regression of colonisation probability for all variables. After backwards stepwise logistic regression, the only variables remaining in the model were patch area and current population connectivity, both of which increased the likelihood of colonisation (Table 3.6). Prior connectivity almost achieved significance, but the Z-score indicated it to have a negative effect on colonisation in this model, which is inconsistent with the previous results and likely to result from a strong correlation between (natural log-transformed) current and prior connectivities (Spearman's r_s=0.783, P<0.001). The model explained just 17% of the null deviance in the data, but observed colonisations were fairly strongly correlated with their model-predicted probabilities (Pearson's r=0.415, P<0.001) and the AUC value was also quite high at 0.785.

(a) Beningbrough Hall



(b) Newton-on-Ouse

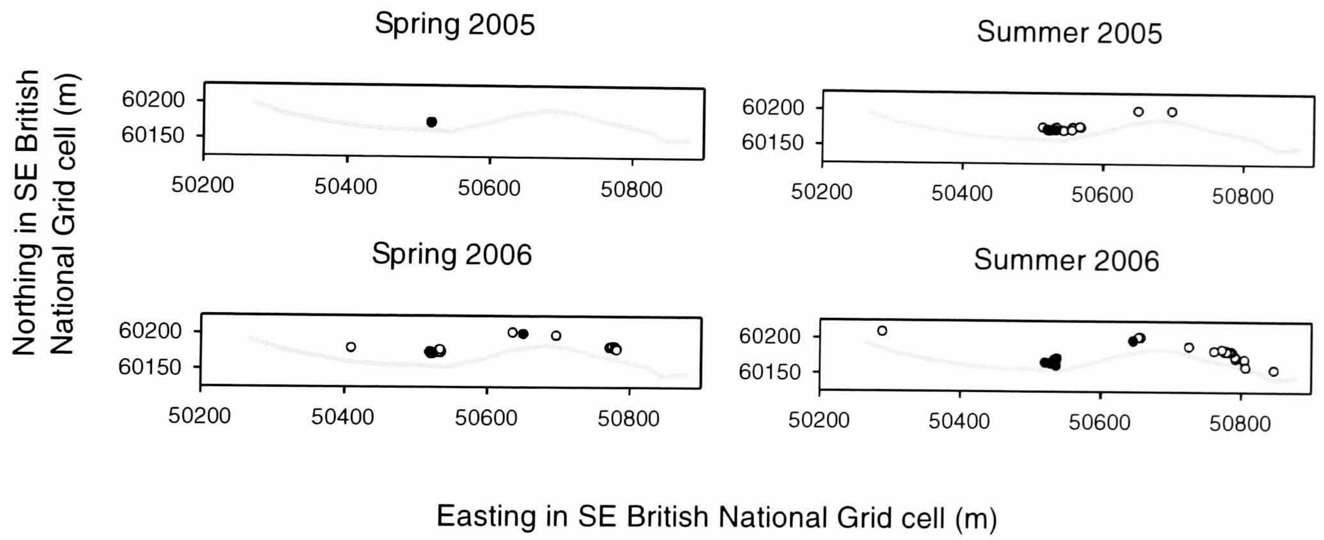


Figure 3.9. Locations of tansy patches at (a) Beningbrough Hall and (b) Newton-on-Ouse, showing the spread of beetles following translocations made in Spring 2005 through time. Black dots show the locations of occupied patches, while white dots show unoccupied ones (these were not surveyed at release so are not shown in the earliest maps). The grey line shows the position of the River Ouse over the extent to which surveying was performed.

Table 3.5. The number of tansy patches that were found, their occupancy rates and the number of beetles censused during each survey.

Survey	Number of patches	Proportion occupied	Number of beetles
Summer 2005	107	0.196	575
Spring 2006	118	0.263	66
Summer 2006	148	0.507	401

Of the six pairwise combinations of continuous variables, significant correlation was only found between (ln) area and height (r=0.597, P<0.001) and current and prior connectivity (see above). Hierarchical partitioning concurred with the former analyses, giving patch area, height and both forms of connectivity significant independent effects and showing that current connectivity had the greatest effect in determining which patches were colonised (Figure 3.10).

Table 3.6. Backward stepwise logistic regression model for the colonisation probability of tansy patches during the expansion of translocated beetle populations.

Variable	**Coefficient (S.E.)**	***Z***	***P***
Intercept	-0.101 (0.248)	-0.406	0.685
Patch basal area (m^2) ^{ln}	0.388 (0.133)	2.922	0.003
Current population connectivity ^{ln}	0.650 (0.134)	4.856	<0.001
Omitted variables			
Maximum patch height (m)		1.139	0.255
Prior population connectivity ^{ln}		-1.861	0.063
Site (Beningbrough Hall or Newton-on-Ouse)		0.853	0.394

^{ln} natural log-transformed

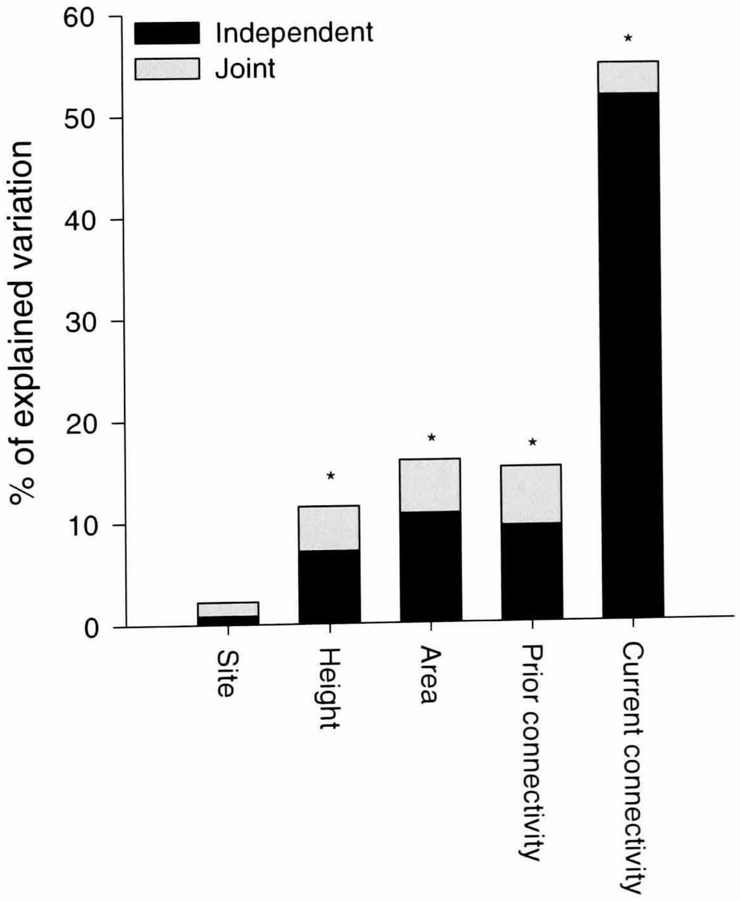


Figure 3.10. Results of hierarchical partitioning of logistic regression models for tansy patch colonisation by beetles following translocation, including predictor variables for the effects of patch size, current and historical connectivity and site (Beningbrough Hall or Newton-on-Ouse). Patch area and both connectivities were log-transformed. Bars are labelled with asterisks where the independent effect was significant (P<0.05).

3.8 Discussion

3.8.1 Conservation status of the beetle around York

Surveys of the beetles' range over the last decade have revealed a regionally stable but locally variable and highly restricted distribution. However, the range has contracted from around Linton-on-Ouse south to Overton at some point in the last 50 years, until translocations were performed as part of this study. Although the apparent stability over the last decade is encouraging for conservation in that York does not appear at imminent risk of losing the species, the small size of the range makes it inherently vulnerable. This is especially so given the potential for range-wide disturbance in the form of flooding. *C. graminis* larvae are very vulnerable to inundation (Sivell 2003), so if a severe flood occurred when larval activity was at a peak the entire British population could be dealt a serious blow.

Anecdotally, many beetle populations appear to fluctuate independently of one another. For example, since the study of Sivell (2003), populations at Fulford Ings and South Ings have dramatically increased in number, while others such as those at Skelton and Middlethorpe Ings have declined. A recent colonisation of the Esplanade section of the river downstream of Clifton Ings was spreading during Sivell's (2003) study, but had declined when the area was visited in 2005. Asynchronous fluctuations may buffer the population against perturbations, as some subpopulations will be at high densities when disturbance occurs and these will be most likely to endure it. Long-term persistence will rely on recolonisation after local extinction, but this may be prevented if the bank contains large gaps in the tansy patch network. Translocations may help to counter this and seem to be a viable option for the conservation of *C. graminis.*

3.8.2 Structure of the tansy patch network

Point pattern analyses were used to show that the patch network inhabited by the beetle around York is characterised by an aggregation of patches with randomly distributed patch areas (except for a negative association within 20 m) and positively autocorrelated heights. An aggregated tansy patch network could be produced by its reliance on disturbance, whereby clusters of tansy develop where, for example, flooding scours the bank and deposits sediment or where human activities such as bank engineering works occur. Nothing is known about the seed dispersal or seed bank of tansy, but if dispersal distances were limited and the seed bank's viability short-lived then such patterns could also be caused by multiple patches springing up close to their parents, while sites far from other tansy plants receive no seed. The small-scale negative autocorrelation in patch areas may relate to competition between plants, but may also be an artefact of the treatment of patches as points, since the centres of small patches can be nearer to the centres of large patches than other large patches, which could generate such a negative autocorrelation. Interestingly, patch heights were positively autocorrelated across all scales. This may partly reflect the way the survey was carried out – in reasonably large blocks of the bank over nearly two months. Thus, sections surveyed earlier in the study may have had shorter patches than those surveyed later because tansy was growing. This is more likely to affect patch height than area, since most tansy growth in spring is vertical.

Given the distribution of tansy patches, beetles were spatially autocorrelated over wide scales (up to 2.7 km). This is approximately three times the known limit of tansy beetle dispersal (see Chapter 5), and is consistent with the autocorrelation in patch height and the strong role of population connectivity in promoting patch occupancy and colonisation, and suggests that rare dispersal events over longer distances than the furthest known may exist. Alternatively, this autocorrelation could be built up over a number of generations and dispersal events. It is also possible that some unknown environmental autocorrelation other than in patch height also acts to bring about this result. However, since I found no substantial effect of patch quality on occupancy it seems that this will be of lesser importance compared to the former processes.

3.8.3 Factors promoting beetle incidence

3.8.3.1 Patch size and population connectivity

Island biogeography and metapopulation theory have long stressed that the balance between colonisation and extinction leads to larger and less isolated islands or habitat patches having greater probabilities of occupancy at any one time (Hanski and Gaggiotti, 2004). For *C. graminis*, this process operates on a smaller scale – within populations on individual host plant patches. Tansy patch size (height, basal area and the number of fronds) and population connectivity (the summed distance to other beetles weighted by dispersal ability) strongly increased occupancy and colonisation probabilities in three sets of survey data. Studies of tansy beetle movement have suggested that in most cases, local inter-patch movement rates are too high for closely situated patches to support independent subpopulations (Sivell 2003; Chapters 4 and 5) so that at the scale of individual host plant patches, *C. graminis* can be considered to have a patchy population structure rather than function as a metapopulation (Harrison 1991; Thomas and Kunin, 1999). Thus, these results show that relationships observed within metapopulation contexts also apply to patchy populations, and at very local scales.

By using a combination of two approaches – stepwise logistic regression and hierarchical variation partitioning – variables that figure significantly in both results can be considered causally responsible for the observed patterns of patch occupancy and colonisation with greater confidence. In the survey of patch size and isolation, logistic regression suggested that tall patches with large basal areas, high population connectivities and low network connectivities were more likely to be occupied. Hierarchical partitioning confirmed this result, suggesting that the most important factor was population connectivity, followed by patch height. Network connectivity contributed a small (but significant) independent effect, but the magnitude of its negative joint influences in suppressing the predictive power of other variables was almost as great as its independent effect, so the independent effect was probably caused by positive correlations with the other variables. It may also be possible to explain why the logistic regression showed a negative effect of network connectivity. Although over the whole system, the network connectivities of occupied and unoccupied patches are statistically indistinguishable (Figure 3.4d), in areas of the bank where tansy forms dense aggregations of large and close patches, patch radii may significantly inflate centre-to-centre inter-patch distances so larger patches have lower network connectivities than smaller ones. Since patch size increases the probability of occupancy, network connectivity may thus come out of the analysis as negatively affecting beetle incidence. A better measurement of connectivity would, in future, utilise edge-to-edge distances but unfortunately the dispersal kernel used here was developed for centre-to-centre distances (Chapter 5) and patch edge-to-edge distances were unknown.

3.8.3.2 Patch quality

There was little evidence that tansy patch quality affected beetle occupancy, either in a core area (Clifton Ings) or an area near the range margin that *C. graminis* had recently re-expanded into (Skelton). Logistic regression analysis identified patch area, population connectivity and tansy groundcover as having positive effects on occupancy while the height of surrounding meadow vegetation had a curvilinear relationship with the optimal height for occupancy being ~0.7 m. There were no significant effects of site (Clifton Ings or Skelton), bank slope or aspect, the number of tansy fronds, the presence of flowering heads in any developmental stage, the hardness of soil below the patch or encroachment by trees and shrubs. Of the significant variables, only sward height and tansy groundcover density can be said to relate to habitat quality. Hierarchical partitioning concurred with the logistic regression in revealing significant independent effects of patch area and population connectivity but gave no significance to groundcover, sward height or any of the other quality variables. The number of fronds was also significant and was probably eliminated from the logistic regression because of its strong correlation with patch area. Patch area and fronds had large joint effects, and it seems likely that these are mainly interactions with each other, given the relatively small joint effects of the other variables. A limitation of hierarchical partitioning is its inability to cope with non-linear responses (Heikkinen *et al.* 2005), which were found for sward height. Although minimised through transformation, this may have reduced the precision of the analysis, so I cannot rule out the possibility that sward height is important for occupancy. Overall however, the inconsistent results of the two complementary analyses suggest that there are no strong effects of patch quality on beetle occupancy.

To reiterate, partitioning and logistic regression analysis survey data combined to powerfully support the notions that tansy patch size and isolation from other occupied patches are the principal factors determining occupancy and that patch quality has little affect. I therefore interpret this in terms of *C. graminis* movement and reproduction, regarding equal-sized patches to be of more or less equivalent quality. In this framework, long-term occupancy at regional scales requires a threshold local density of tansy patches above which the burden of mortality in the inter-patch matrix is less than the reproductive rate (Bascompte *et al.* 2002). Large and tall patches will facilitate persistence as low perimeter-to-area ratios will reduce emigration rates (Englund and Hambäck, 2004) and bigger patches will present greater targets to individuals searching for tansy (Byers, 1996) (whether or not movements are random or directed) and support large and less extinction-prone populations (Hanski and Gaggiotti 2004).

There are many potentially important aspects of habitat quality that were not measured directly in the field. *T. vulgare* is known to form at least 30 chemotypes across its range, each of which is defined by the exact array of defensive chemicals it contains. However, within a local area it is thought that one chemotype dominates (Keskitalo *et al.* 2001), so it is likely that *C. graminis* experiences only one form of tansy around York. Other potentially important habitat quality variables not recorded include leaf protein nitrogen levels (Bernays and Chapman, 1994; Obermaier and Zwölfer, 1999) or soil nutrient and water content (Lower *et al.* 2003). However, no study can record or assemble data on every possible environmental variable and the influence of these should be felt through correlation with the variables that were measured. For example, if water regimes affected occupancy then variables such as soil hardness or patch position (riverside or bank-top) might have had significant effects. Similarly, shading is known to increase leaf nitrogen content relative to carbon-based defensive compounds, which may make the leaves more suitable for herbivorous insects (Scriber and Slansky, 1981). If this mattered for *C. graminis*, encroachment by trees, bank slope or aspect may have attained significance.

3.8.3.3 Network connectivity

Network connectivity did not differ between occupied and unoccupied patches, while occupied patches were much better connected to the beetle population than empty ones. Tansy is a plant of disturbed ground with high annual turnover that relies on disturbance to propagate new seed. Therefore the current distribution of beetles will be a product both of the existing patch network and its history (Biederman 2004). If the rate of patch turnover were low relative to colonisation rates, then beetles should aggregate on sections of the bank with high network connectivities. However, more limited dispersal may result in a lag between the development of network connectivity within a region and the beetle's distribution tracking it. Thus the differences in network connectivity between occupied and unoccupied patches may be reduced because of dispersal limitation. As a consequence of this, there will be areas within the current range where the patch network is potentially able to support beetles but where none are found. Thus, translocations could aid in the species' conservation within its current range margins as well as beyond them.

3.8.3.4 Bank management

Where the bank was managed by mowing, beetles were most common. Wooded bank and areas used for amenities (such as yachting) or livestock grazing were less suitable. Given a limited influence of patch quality, it seems that this is because of an influence of the management on the patch network structure. Over-grazing of the bank and the development associated with amenities are likely to reduce the overall size and density of tansy patches, while tansy will rarely grow successfully in wooded areas because of heavy shading and low disturbance. On the other hand, mowing is likely to provide sufficient disturbance to encourage tansy growth and reproduction. Although network connectivity has no effect on patch occupancy over the whole network, those areas that have historically been managed by mowing are likely to have maintained dense clusters of tansy patches over long periods of time. Temporal stability in the patch network is likely to promote occupancy by the beetle, as regional population sizes will be more stable and there will have been a long time for the beetle to colonise the area. A similar process may operate for the chrysomelid *Gonioctena olivacea*, for whom modelling work suggested that when its host plant network is more stable, the expected time to extinction is higher (Biederman 2004).

3.8.4 Factors promoting patch colonisation

Following translocations, connectivity to the current population was a better statistically predictor of colonisation probability than population connectivity to patches recorded as occupied several months before. This would be the expected pattern if colonisation resulted from multiple dispersal events occurring continually through the year rather than being the product of a single specific dispersal period in which dispersers originated from patches recorded as occupied previously. Colonised patches were also taller and of larger areas than those remaining empty. All these patterns are consistent with those found in the occupancy surveys described above, and would result from dispersal being biased towards large patches or if the probability of gravid female colonists founding a population through oviposition was higher in larger patches.

The fact that beetles survived and dispersed from five release sites suggests that there is nothing inherently unfavourable about the areas around Beningbrough Hall or Newton-on-Ouse. Thus, the extinctions that occurred there in the second half of the 20th century probably either resulted from temporarily unsuitable conditions (e.g. over-grazing) or were stochastic events. Recolonisation probably could not occur because of barriers to normal dispersal such as wooded sections of bank and because the area is upstream of other beetle populations so could not receive any water-borne flood dispersers.

3.8.5 Conclusions

Although the beetle's range around York has contracted in the last century, it appears to have been regionally stable (albeit with fluctuation at the margins) for the past decade. However, even though it is not currently in decline, the small extent of the range in itself increases the beetle's vulnerability and necessitates conservation action. Translocations may be a successful strategy for overcoming bank fragmentation and poor dispersal ability, as is suggested by the similar network connectivities of occupied and unoccupied patches and the success of translocations to expand the beetle's range. Management of the patch network should focus on creating clusters of large patches, which may need the introduction of beetles depending on their location.

In occupancy-environment studies I contend that it is important to study environmental effects on a spatial scale relevant to the behaviour of the organism. In this chapter, I asked whether the characteristics of individual tansy patches influence their occupancy by tansy beetles or the probability of beetles colonising them and found that both were more likely in large and less isolated patches. However, there was no convincing evidence of an effect of patch quality on occupancy. *C. graminis* inhabits an aggregated patch network although beetles are aggregated over and above this, probably due to dispersal limitation.

Chapter 4: Movement behaviour

4.1 Introduction

Animal movement is ecologically important at all scales. Movement is a mechanistic element in the foraging choices, habitat selection, home ranges and dispersal of individuals, the dynamics and distributions of populations, and the coexistence and life history evolution of species (Aars and Johannesen 1999; Clobert *et al.* 2004; Ronce and Olivieri 2004; Bowler and Benton 2005). As such, movement models are integral components of broader models in spatial ecology.

One way to model movement is by using correlated random walks to simulate habitat-dependent paths through heterogeneous landscapes (Tischendorf 1997). This seems reasonable since wide-scale phenomena such as population redistribution arise from the behaviour of individual organisms, so models based on individual behaviour should extrapolate to higher organizational levels and capture the essence of patterns observed at these scales (Turner *et al.* 1989; Lima and Zollner 1996; Grimm and Railsback 2005). However, individual movement decisions may be based on numerous factors such as habitat quality, physiological condition, previous experience or inter- and intra-specific interactions (McIntyre and Wiens 1999a; Morales and Ellner 2002; Berggren 2005) and may operate on multiple scales (Nams 2005). Also, movement may not be random, either as individuals employ a systematic strategy, such as looping (Bengtsson *et al.* 2004) or 'foray search' (Conradt *et al.* 2001, 2003), or if the animal can detect and move towards the resources it is seeking (Müller & Hilker 2000, 2001; Schooley and Wiens 2003). This raises the question of whether or not simple models of fine-scale behaviour can produce realistic patterns of wider-scale movement in real landscapes.

Animals adapt their movement behaviour to their surroundings, so this at least must be accounted for when attempting to address this question (Stevens *et al.* 2004). In unfavourable habitat, many organisms increase net displacement by travelling in less tortuous paths and increasing their step lengths (Kindvall 1999; Banks and Yasenak

2003), which is an efficient way of locating resources (Zollner and Lima 1999). A consequence of habitat-dependent behaviour is that movement rates through differently configured landscapes will vary. For example, the movement of three species of *Eleodes* beetle differs between vegetation types, with higher net displacements on bare ground and grass than on cactus or scrub (Crist *et al.* 1992). For *E. obsoleta* this results in a stronger response to environments of intermediate-sized habitat patches than to fine- or coarse-grained microlandscapes (McIntyre and Wiens 1999b).

A further complication to simulating movement in heterogeneous landscapes is that animals may alter their behaviour when reaching the boundary between habitat-types. Commonly, individuals will choose not to leave patches of favoured habitat, or turn back immediately after doing so, rendering the patch-edge semi-impermeable (Stamps *et al.* 1987; Schtickzelle and Baguette 2003). This affects movement at wider scales by limiting dispersal rates, creating area-emigration relationships and causing “fat-tailed” distributions of movement distances (Schtickzelle and Baguette 2003; Morales 2002). Conversely, individuals just outside a habitat patch may have an increased chance of crossing the boundary from unfavourable to favourable habitat if they are able to detect and move towards the resources they are searching for (Ovaskainen and Cornell 2003; Ross *et al.* 2005). This will increase the proportion of successful inter-patch movements but could decrease their overall rate because recent emigrants may re-enter their source patch. Thus, boundary-mediated behaviour will also be a part of realistic simulations of landscape-level movements.

The tansy beetle provides a model system for testing whether a simple fine-scale movement model can be scaled up within real landscapes, which is the main aim of this chapter. Initially, beetle movement behaviour and sensory abilities are examined through laboratory choice experiments and observational studies in semi-natural settings. A habitat-dependent correlated random walk simulation for movement of the beetle within and between tansy patches is then constructed. The model is based upon observed paths on both tansy and matrix meadow vegetation and includes semi-impermeable patch boundaries. Simulations of movement within maps of real landscapes are compared to patterns of daily movement quantified independently in the field during two mark-resight studies to ask whether landscape-level movements emerge from the simple behavioural model.

4.2 Orientation towards tansy

A number of experiments were performed to determine whether or not adult and larval tansy beetles are able to detect and move towards their host plant tansy from a distance. These are outlined below.

4.2.1 Attraction of adult beetles to tansy by olfaction

4.2.1.1 Materials and Methods

Tansy leaf volatiles were extracted in hexane with a procedure based on one previously used to demonstrate the orientation of other chrysomelid beetles towards tansy (Müller and Hilker 2000). Fifty grams of freshly picked tansy leaf tissue were ground in liquid nitrogen and added to one litre of hexane. The mixture was left overnight in a sealed flask with a stirring bead before the tansy particles were filtered out through a double layer of muslin. The extracted solution was then concentrated to 100 ml in a rotary evaporator at 15°C with cooling at 4°C, resulting in a liquid that smelt strongly of tansy.

To test whether adult beetles would orient themselves towards the tansy extract, a 'crucifix' was constructed from bamboo skewers (20 cm high with a 20cm horizontal arm fixed 1.5 cm from the top). This was planted into 5 cm of soil in a high-sided tank that shielded it from any air currents. Balls of cotton wool were attached to the end of each arm with wire. To prevent beetles seeing the cotton wool from the centre of the crucifix, 4 cm diameter discs of filter paper were attached 4 cm in from the end of each arm. The entire apparatus was lit from above with a lamp.

Bioassays were performed by pipetting tansy extract (0.25, 0.5 or 1.0 ml) onto one of the cotton wool balls and an equal volume of hexane control onto the other. The apparatus was left for 2 minutes to allow the hexane to evaporate and a tansy odour gradient to develop (which could clearly be smelt in the tank). Beetles from a captive population housed at the University of York Walled Garden (starved for three hours prior to the assay) were then introduced singly to the base of the arena, from where they would climb to the top of the cross. After trying unsuccessfully to leave the top of the cross they walked back down until hitting the horizontal arms, one of which would be chosen and its identity recorded. In each bioassay, groups of ten beetles were tested 20 times each, with 180° rotation of the arena and reapplication of the tansy extract after each round of trials to destroy any directional bias caused by, for example, exterior light sources.

4.2.1.2 Results

The 'crucifix' experiment gave a dataset of the number of times out of 20 that the tansy arm was chosen over the control by 30 beetles. If beetles were making random choices, these data should follow a binomial distribution with a probability of 0.5. To test this, the expected and observed cumulative distributions of the numbers of times the tansy arm was picked were calculated. The maximum difference between these was found and converted into the Kolmogorov-Smirnov test statistic, $Z = D_{\max} + (2n)^{-1}$, where $D_{\max}$ is the maximum absolute difference between observed and expected and n is the sample size (Sokal and Rohlf 1995). This showed that there was no overall significant preference for beetles to pick the tansy arm over the control or vice versa (Z=0.068, P>0.05), indeed they were remarkably close to the random expectation (Figure 4.1).

Although there was no overall preference, beetles experiencing different concentrations of tansy extract or from different sexes may have behaved differently and averaged out to give no preference for tansy. Since the data followed a binomial distribution overall, the repeated observations of the same individuals can be treated as independent data points, giving a dataset of the number of times out of 600 that tansy was chosen over the control. Differences in the choices made in the concentration treatments or by each sex were assessed with χ^2 tests, showing no effect of tansy concentration (χ^2_2=0.220, P=0.900) or differences between sexes (χ^2_1=0.167, P=0.682).

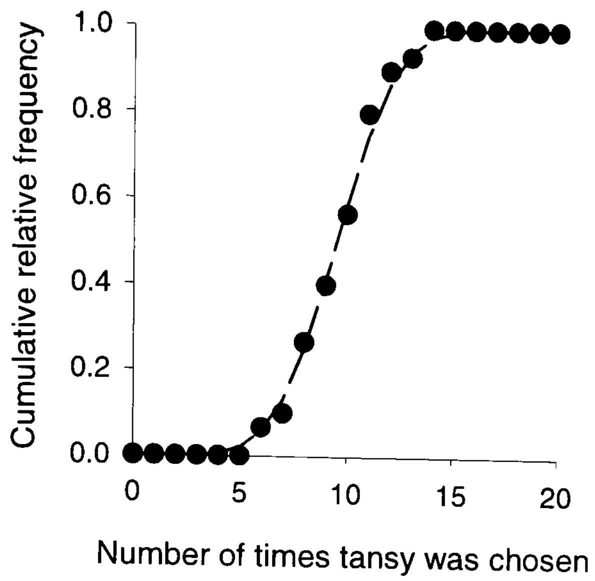


Figure 4.1. Cumulative frequency distributions for the observed (circles) number of times out of 20 that the tansy arm was chosen over the control and that expected from a binomial distribution with a tansy-choosing probability of 0.5 (dashed line).

4.2.2 Detection of tansy by beetle larvae

4.2.2.1 Materials and Methods

Two experiments were performed to assess whether tansy beetle larvae can detect cues from tansy. The first was based on those of Müller and Hilker (2000, 2001) and was designed to test whether or not larvae can use olfaction to orient towards tansy. A petri dish (14 cm diameter, 3 cm depth) was filled with soil to a depth of 2 cm, upon which a 13 cm diameter blotting paper disc was placed. 'Stem dummies' were created by cutting 5.5 cm lengths of bamboo skewer and wrapping one end of them with a 3 cm long freshly picked tansy leaflet or a ground elder *Aegopodium podagraria* leaf. Ground elder was chosen to represent a non-tansy matrix species as it is found at the riverbank and there was a plentiful supply of it at the University of York Walled Garden. Leaf material was fixed to the bamboo with wire.

Three tansy and three ground elder stem dummies were planted in alternate order around the circumference of a 3 cm diameter circle in the centre of the petri dish's blotting paper disc at even intervals. Each of these were covered with a sheath made from perforated blotting paper (5 cm high with 10 perforations per cm^2 made with a

1 mm diameter dissecting needle) to block any visual cues but allow olfactory cues to pass (the odour of tansy was evident through the sheath).

Groups of 11-25 one day old, first instar larvae (hatched in the laboratory from wild-collected eggs) were starved for three hours and then released into the central circle of stem dummies. The numbers of larvae on tansy and control stems were recorded every 30 seconds for 15 minutes after the release. Ten replicates were performed at 24-25 ^{o}C in a room with no external lighting and a lamp placed directly overhead.

A second experiment was designed to test whether larvae could detect tansy at a distance and also whether they adapt their behaviour when on tansy. Four petri dishes (9cm diameter) were lined with blotting paper. In each dish two leaf discs (18 mm diameter, cut from freshly picked leaves of either tansy or ground elder with a metal corer) were placed 2 cm apart with plant species varying between dishes. Groups of 5-10 one day old, first instar larvae that had been starved for three hours were released onto one of the leaf discs (the release disc) and the number of larvae on the release disc was monitored every minute for 20 minutes. The four treatments were (release disc-other disc) tansy-tansy, tansy-ground elder, ground elder-tansy, ground elder-ground elder. Seven replicates of each treatment were performed in a room with no external light source.

4.2.2.2 Results

In the stem dummy experiment a maximum of 53% of the larvae were climbing a stem at any one time. The proportions of released larvae on each of the two stems in each replicate were compared with repeated measures ANOVA (with a Huynh-Feldt correction to the degrees of freedom to account for non-sphericity in the error covariance matrix). The repeated measures part of the ANOVA showed a significant effect of time ($F_{4.42,\ 79.49}$=27.3, P<0.001), but no interaction between time and the stem dummy type (tansy or control) ($F_{4.42,\ 79.49}$=1.67, P=0.159). The non-temporal part of this test showed that there was no significant preference for the tansy stem dummy over the control ($F_{1,\ 18}$=0.244, P=0.627) (Figure 4.2a), which suggests that larvae move randomly with respect to odour cues from their host.

The results of the leaf disc experiment were also analysed with Huynh-Feldt corrected repeated measures ANOVA taking the identity of the release and other discs as factors. Time had a significant effect on the proportion of larvae on the release disc ($F_{3.46,83.06}$=49.0, P<0.001; Figure 4.2b) and significantly interacted with the identity of the release disc ($F_{3.46,83.06}$=9.81, P<0.001), but not the other disc ($F_{3.46,83.06}$=0.564, P=0.665) and there was no significant three-way interaction ($F_{3.46,83.06}$=0.278, P=0.867). In the non-repeated part of the ANOVA, the identity of the release disc significantly affected emigration from it ($F_{1,24}$=26.42, P<0.001; Figure 4.2b) but the other disc did not ($F_{1,24}$=0.695, P=0.413) and there was no interaction between the two ($F_{1,24}$=0.412, P=0.527). This suggests that larvae could tell when they were on tansy but could not detect it at distance. Larvae on the tansy discs slowed their movement, either by reduced activity or by feeding, resulting in slower loss of larvae from the tansy disc.

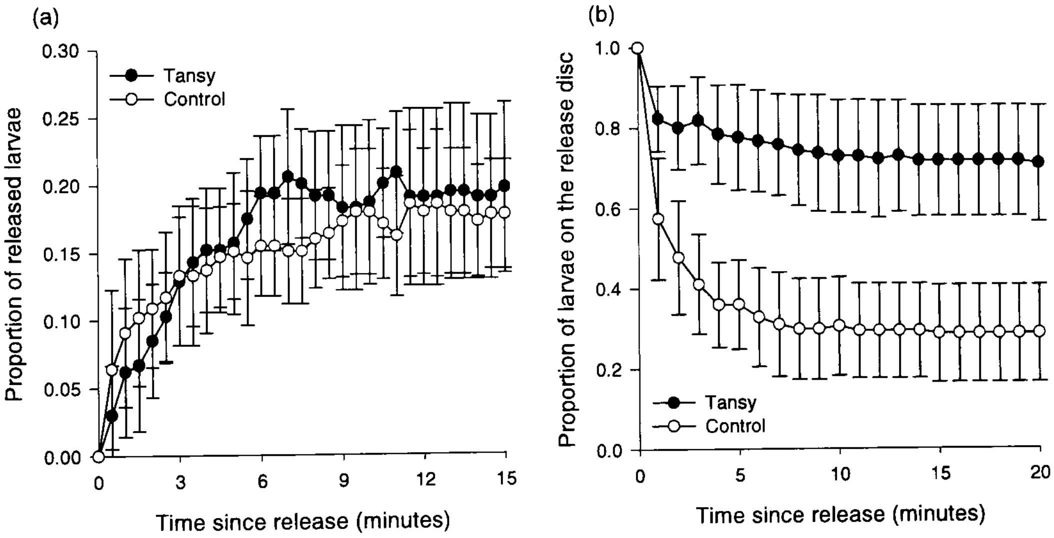


Figure 4.2. (a) Plot of the mean proportion of released larvae in each replicate on tansy and ground elder control stem dummies through time with 95% confidence intervals. (b) Mean proportion of larvae on tansy and control release discs through time with 95% confidence intervals.

4.3 Temporal activity patterns

4.3.1 Materials and Methods

A simple experiment was performed to test whether or not *C. graminis* activity levels change throughout the day. Adult beetles were collected from a captive population housed at the University of York Walled Garden (established from beetles sourced throughout the beetle's York range) on the afternoons of 30th May (N=29) and 5th June (N=59) 2006. On each afternoon, beetles were divided haphazardly among 10 petri dishes. Each dish was provisioned with a freshly picked tansy leaf and placed in a weedy flowerbed. The dishes were left for one hour to allow the beetles to acclimatise and were then inspected at intervals over the next 24 hours, recording the number of beetles moving at the instant of inspection. The null hypothesis that activity levels do not differ between the night and day was tested with χ^2 tests.

4.3.2 Results

During the course of the experiment, two beetles died on the afternoon of 31st May and 16 died on the afternoon of 5th June. The high number dying in the second trial was probably due to a 'greenhouse effect' within the petri dishes raising the temperature to lethal levels. Because so many were dying, the experiment was stopped at 2 pm on June 5th. The number of individuals moving at each inspection varied significantly ($\chi^2_{16}=182.0$, $P<0.001$). Splitting the observation periods into night and day revealed that beetles were significantly more active in the day than the night ($\chi^2_1=98.78$, $P<0.001$) (Fig 4.3).

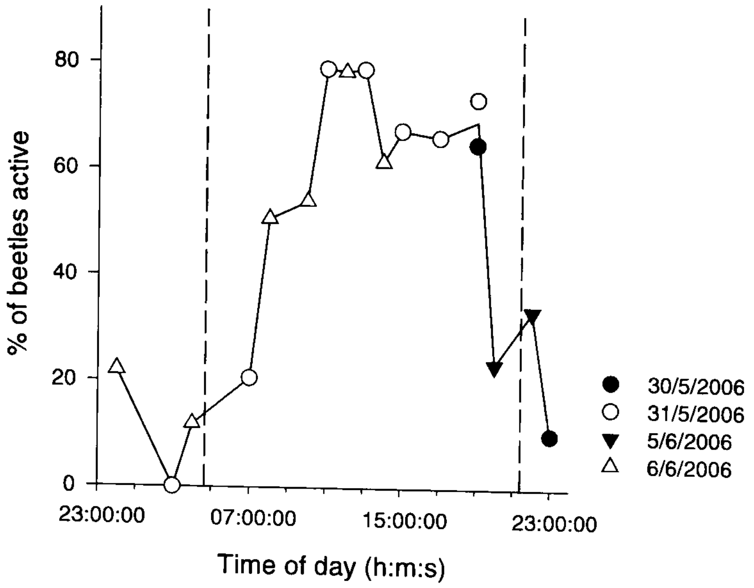


Figure 4.3. Temporal pattern of beetle activity levels shown as the proportion of surviving beetles that are active through time. The vertical dashed lines show the times of dawn and dusk, used to divide the observation periods into night and day categories. Activity was significantly lower at night than in the day (see main text).

4.4 Effects of hunger and conspecifics on movement rate

4.4.1 Materials and Methods

It is possible that while being unable to detect and orient towards tansy, beetles may be able to respond to cues from their conspecifics (or cues produced by tansy in response to beetle herbivory). Since beetles are at higher densities on tansy, it could be adaptive for beetles in the matrix to move towards other beetles, as this would help them locate food and mating opportunities. It is also possible that hunger interacts with beetles' abilities to locate food or movement speed, as hungry individuals should have greater motivation to find tansy.

To see if this was the case, an experiment was performed with wild-caught beetles that had been starved in the laboratory for 0, 24 or 48 hours. Before starvation, beetles were marked with three dots using an engraving drill (see Chapter 5) to show which starvation group they belonged to. After starvation, 25 beetles from each of the three groups were divided equally among five petri dishes. These dishes were taken to a plot of meadow vegetation in the Walled Garden bounded on either side by plots of tansy (Figure 4.4). The beetles were released into the centre of the plot by removing the dish lids.

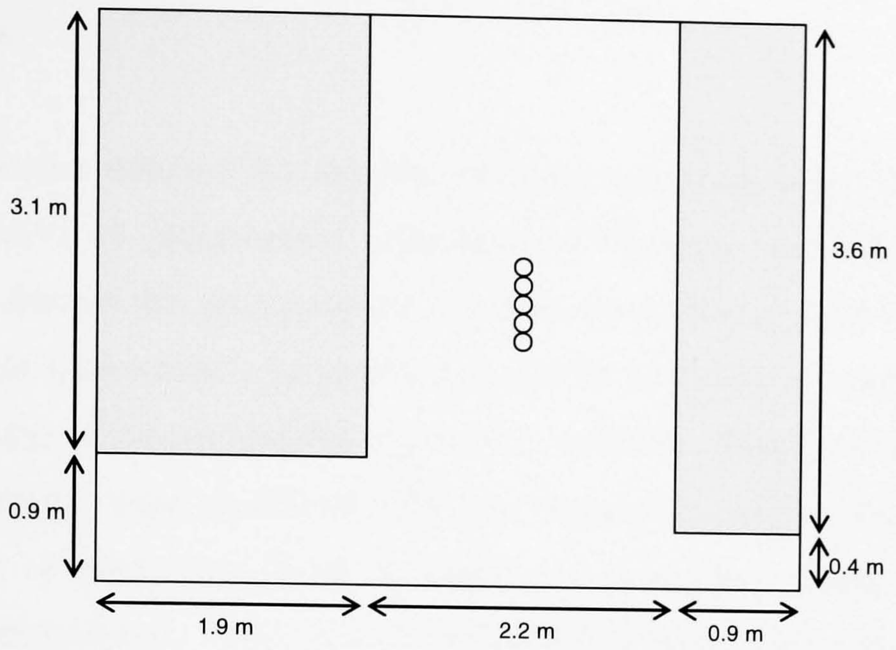


Figure 4.4. Scale drawing of the plot used in the experiment to assess whether beetles moved differently when hungry or oriented themselves towards their conspecifics. The hatched rectangles show tansy patches within tansy-free meadow vegetation represented by the white box. The entire plot was surrounded by bare ground and paving. Beetles were released from five petri dishes (circles, not to scale), each containing 15 beetles, in the centre of the plot.

Two days prior to each release, the plots were prepared by pulling up any tansy shoots growing in the meadow section (this was also done on the day of every release although almost all tansy was removed in the first weeding) and by fixing 19 adult beetles to one of the tansy patches. Ten of these beetles were attached singly to the tansy in clear plastic bags, perforated with holes to allow the passage of air, which were fixed to the end of a prominent stem with wire ties. The other nine beetles were placed in mesh nets stapled to the end of prominent tansy stems. Thus any visual or olfactory cues emanating from these beetles or from the tansy in response to their herbivory should have been produced. On the other tansy patch an equal number of empty bags and nets were attached in the same manner.

Four replicate releases were performed, with beetles attached to each patch twice. The patches were monitored continuously for four hours, collecting the beetles arriving at each patch and noting the time they took to arrive, their sex, starvation group and the patch identity. Preferences for moving towards the larger patch or that containing their conspecifics were tested for by analysing the overall frequencies of beetles recovered.

4.4.2 Results

Of the 300 beetles released into the plot, 107 were recovered during the observation periods. *G*-tests of independence adjusted with Williams' correction (Sokal and Rohlf 1995) showed that starvation did not effect the choice of patch with respect to whether or not it contained conspecifics (G_2=0.978, P=0.613) or whether it was the larger or smaller of the two patches (G_2=2.915, P=0.233). Therefore the data for the starvation groups were combined and the frequencies of beetles reaching all combinations of patch size (large or small) and occupancy (empty or occupied) tested for independence. There was no evidence that these were related (G_1=0.091, P=0.763). Thus, the data were combined to test for any outright preference for the large patch or the occupied patch using χ^2 tests, calculating the expected values if both patch types were equally attractive. There were no significant preferences for the larger patch (χ^2_1=0.084, P=0.772) or for the occupied patch (χ^2_1=0.234, P=0.629). Therefore, there was no evidence for beetles orienting their movement towards larger patches or conspecifics and hunger did not induce them to develop any such preference.

The patch arrival times of the three starvation treatments were significantly different according to a one-way ANOVA on these ($F_{2,\,104}$=8.892, P<0.001). Beetles that had been starved reached the patches quicker than those that had recently fed and starvation for 24 hours caused faster arrival than starvation for 48 hours (Figure 4.5). This suggests that beetles increase their movement rate as they get hungrier, as their motivation to find food is higher, but that as they are deprived of food for longer, their energy reserves are depleted and they are unable to maintain this increased movement rate.

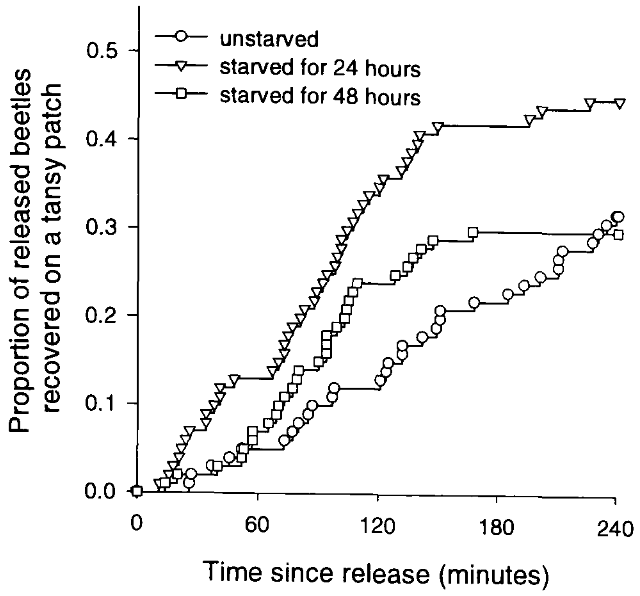


Figure 4.5. Arrival rates to the tansy patches through time for beetles that had been starved for different amounts of time prior to release.

4.5 Movement paths over one hour

4.5.1 Materials and methods

To compare beetle movement inside and outside tansy patches and to provide data for a random walk model, beetles were released and observed moving in two rectangular plots representing the patch (2.0 x 1.2 m of dense tansy cut to ground level and allowed to re-grow to a height of ~20 cm in the University of York Walled Garden) and the inter-patch matrix (2.0 x 2.0 m of ~5 cm high wet meadow vegetation near the University of York Observatory) between 10 am and 5 pm during July and August 2005. In each plot, a grid of thin canes was planted at 20 cm intervals. Beetle positions were recorded by noting their grid cell and estimating their coordinates within that cell to the nearest centimetre. This was done every minute for one hour or until the beetle left the plot, whichever occurred first. To test the accuracy of the location method, a plastic disc (1 cm diameter) was placed in a cell and its position was estimated and then measured. This was repeated 50 times to compare estimated and measured coordinates.

The movement paths of 25 beetles were recorded in the matrix plot, of which 20 were also observed in the patch. The order of releases was randomised with a maximum interval of a week between releases of the same individual. Sample sizes differed between plots because movement in the matrix was more variable and more beetles left the plot so extra data were needed. Movement frequencies, step lengths (net displacements when movement occurred) and turning angles (differences between successive movement bearings) were calculated from the resultant movement paths. Resampling was carried out to find the finest temporal resolution at which consecutive movements in each environment were not correlated in length or turning angle, giving a dataset of independent movements.

To provide covariates for the analysis of these data, beetles were sexed and weighed prior to release and their age was determined as either 1 year (hard elytra, collected before the mass eclosion of the new generation) or 0 years (soft elytra when collected, but allowed to harden before observation). Plot temperature at vegetation height in sunlight during the experiment was also recorded.

4.5.2 Results

Linear regression of the estimated against measured coordinates of plastic discs showed that the estimation of beetle positions was accurate and unbiased (N=50; x-coordinate, Estimated = -0.21 + 1.04xObserved, $F_{1,48}=2127$, $P<0.001$, $R^2=0.978$; y-coordinate, Estimated = -0.60 + 1.04xObserved, $F_{1,48}=3817$, $P<0.001$, $R^2=0.988$). The finest scale at which consecutive step lengths were not significantly correlated was two minutes in the patch (N=132, $r_s=0.097$, $P=0.269$) and five minutes in the matrix (N=73, $r_s=0.193$, $P=0.101$). Because these resolutions were different, further analyses were conducted separately for each habitat-type. The resampled paths yielded 598 observations from the patch plot and 228 from the matrix, giving 193 step lengths and 132 turning angles in the patch and 107 steps and 73 turns in the matrix.

Beetles moved in 32.3% of the 2-minute periods in the patch and 46.9% of the 5-minute periods in the matrix. ANCOVA on individual movement frequencies (weighted by the number of observations for that beetle) with age and sex as fixed factors and weight and temperature as covariates showed that older individuals moved less often in the matrix ($F_{1,19}$=4.83, P=0.041). However, no other main and interaction terms achieved significance.

Log-transformed step lengths from each environment (Figure 4.6) were analysed with ANCOVA using the same factors as above but with the degrees of freedom reduced to the number of beetles minus one to account for multiple observations of the same beetles. No main effects or interaction terms were statistically significant, although there was a trend for reduced step lengths of older individuals in both plots, which was consistent with the result for movement rates.

The turning angles of consecutive movements were not correlated in either plot (circular rank correlations [Batschelet 1981]; patch, N=93, r^2=0.016, P>0.4; matrix, N=46, r^2=0.010, P>0.8). Excluding turns of 0 and π radians for which directions (clockwise or anticlockwise) could not be ascertained, there was no autocorrelation in turning direction (G-test of independence; patch, N=86, G_1=2.265, P=0.132; matrix, N=43, G_1=1.084, P=0.298). Although made over different timescales, turning angles in the two plots were significantly different (two-sample Kuiper's test [Batschelet 1981], $k_{73,\ 132}$=2645.5, P<0.05) with more constrained turning in the matrix leading to a distribution more concentrated around straight ahead (Figure 4.7). The turns made by males and females were statistically indistinguishable (two-sample Kuiper's tests; patch, $k_{48,84}$=648.0, P>0.05; matrix, $k_{25,48}$=402.0, P>0.05).

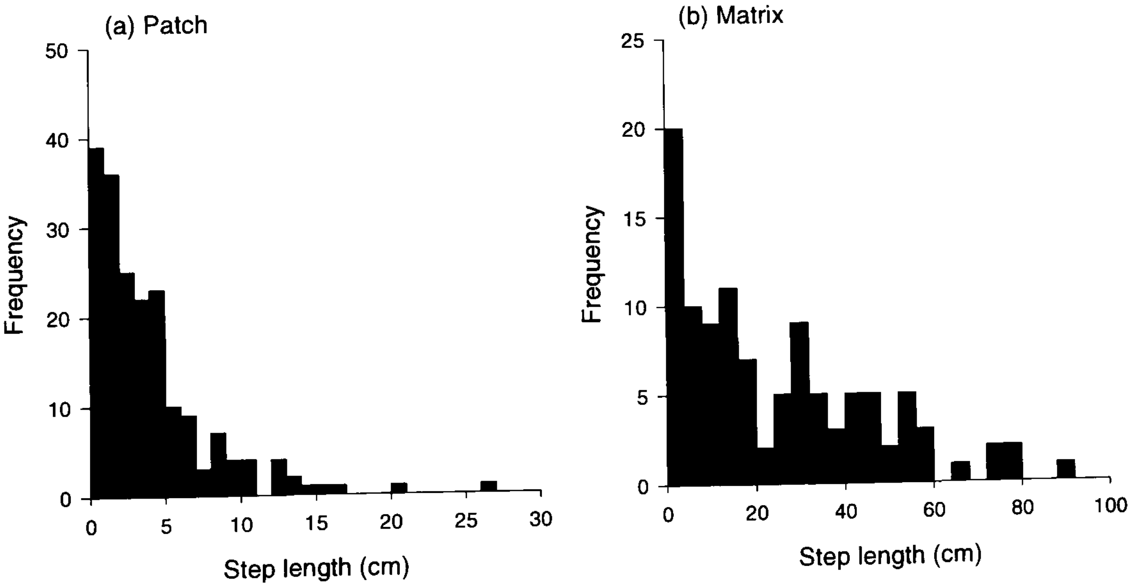


Figure 4.6. Distributions of step lengths in the (a) patch and (b) matrix plots at temporal resolutions of two and five minutes, respectively.

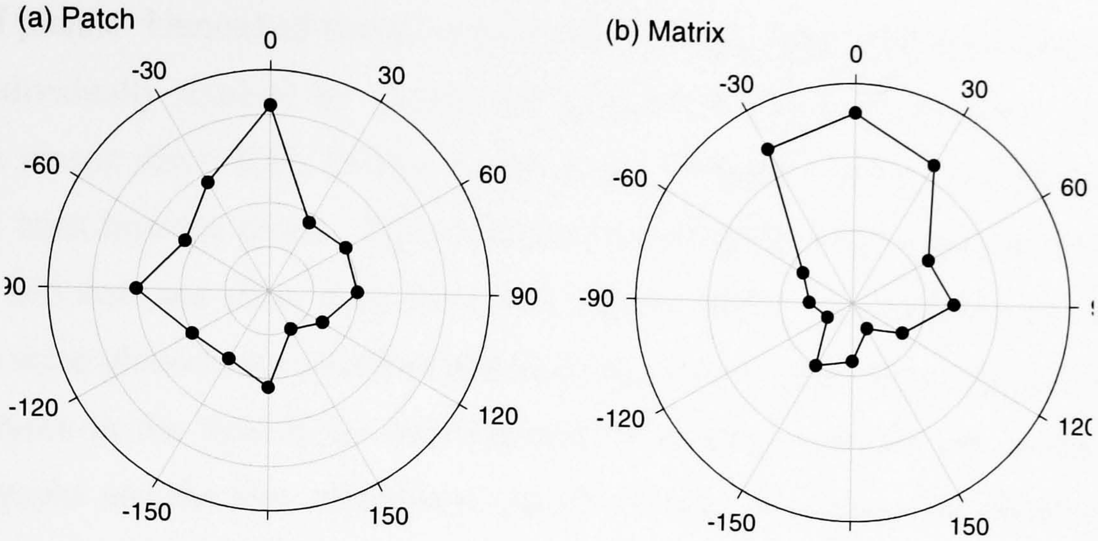


Figure 4.7. Distributions of turning angles (degrees clockwise) made in the (a) patch and (b) matrix plots in 30° categories. Spoke length shows the frequency, with circles every five turns.

4.6 Mark-resight study of movement at daily timescales

4.6.1 Materials and methods

To measure movements over daily time scales, mark-resight (recapture) studies were carried out on a network of 12 tansy patches from 17-30 April 2003 and on a network of 14 patches between 25 May and 1 June 2004 on the same part of the bank of the River Ouse at Skelton, north of York. In this area of the bank, tansy grows in roughly elliptical patches surrounded by a matrix of short meadow vegetation, similar in composition to that in which movement paths were recorded. The patches are bounded by tansy-free scrub and woodland for >200 m on either side, meaning that the patch networks were semi-independent of other patches. The site is approximately 40 m long and 15 m wide with the maximum inter-patch distance being ~30 m in both years. Patch lengths and widths were measured, allowing basal areas and perimeters to be estimated as those of ellipses of these dimensions. Mean patch areas and perimeters were 3.53 m^2 and 6.59 m in 2003 and 1.83 m^2 and 4.57 m in 2004.

In 2004, the site was visited every morning, while in 2003 visits were made in both mornings and afternoons, except during rain when beetles spend less time near the tops of plants. Unmarked beetles were collected from tansy patches by hand, sexed and individually marked by gluing two queen-bee tags (coloured and numbered convex plastic discs; E.H. Thorne Beehives Ltd, Wragby, UK) to their elytra before release back into the patch. This method of marking has been used on the species before and does not affect their behaviour (Sivell 2003). The positions of resighted beetles were plotted onto overhead photographs of each patch, allowing their relative coordinates in the field to be determined by converting between the scales of the photographs and the site. Individual fronds of tansy were generally identifiable in the photographs, providing a sufficiently precise location method. A note was also made if beetles were mating when resighted. The inter-patch matrix was not searched as beetles were at low densities and not apparent there.

Net displacements between resightings separated by non-overlapping whole day intervals were calculated from the coordinate data. Inter-patch movement rates were calculated as the proportion of beetles resighted at both the start and end of the interval that had moved into a new tansy patch. Capture histories from the entire site were entered into the 'Recaptures only' module of MARK (White & Burnham 1999) and daily survival and detection probabilities were estimated as time-invariant parameters.

4.6.2 Results

In 2003, 255 beetles were marked and, on average, resighted 6.3 times each, while in 2004, 207 beetles were marked and resighted an average of 3.2 times each. Estimated daily mortalities (with 95% confidence intervals) were 4.51% (3.58-5.67%) in 2003 and 5.86% (4.36-7.83%) in 2004, giving effective life expectancies (the time for 50% to die or otherwise be lost from the system) of ~15 days in 2003 and ~11 days in 2004. The data contained 1458 net displacements from 2003 and 542 from 2004, over intervals of up to 13 and 6 days, respectively, thus encompassing a large proportion of the effective life expectancies. Daily displacements were right-skewed (skewness of 4.15 in 2003 and 2.46 in 2004) and highly leptokurtic (kurtosis of 22.0 in 2003 and 6.98 in 2004) and daily inter-patch movement rates were 15.6% in 2003 and 9.6% in 2004.

Net displacements were normalised with log-transformations and subjected to ANOVA (N=2000) with the degrees of freedom reduced to the number of beetles minus one to account for repeated observation of the same individuals. Net displacements were greater in 2003 than 2004 ($F_{1,462}$=8.65, *P*=0.003), and over longer intervals ($F_{12,462}$=14.0, *P*<0.001) (Figure 4.8a). However, there were no significant effects of sex ($F_{1,462}$=0.44, *P*=0.510) or mating ($F_{1,462}$=0.01, *P*=0.910). No two-way interaction terms were significant except for that between year and sex ($F_{1,462}$=4.66, *P*=0.032), with marginally further movement by males in 2003 and females in 2004.

The factors affecting the likelihood of moving between patches were analysed with forward stepwise logistic regression (N=2000), using log-transformations to improve the normality of continuous variables. This indicated that inter-patch movement was more common in 2003 than 2004 (Wald_1=49.2, *P*<0.001) and over longer intervals (Wald_1=167.1, *P*<0.001) (Figure 4.8b). High patch perimeter-to-area ratios (log-transformed) also promoted inter-patch movement (Wald_1=26.4, *P*<0.001). However there were no independent effects of (log) patch area (Wald_1=0.544, *P*=0.461), sex (Wald_1=1.35, *P*=0.245) or mating (Wald_1=0.016, *P*=0.901).

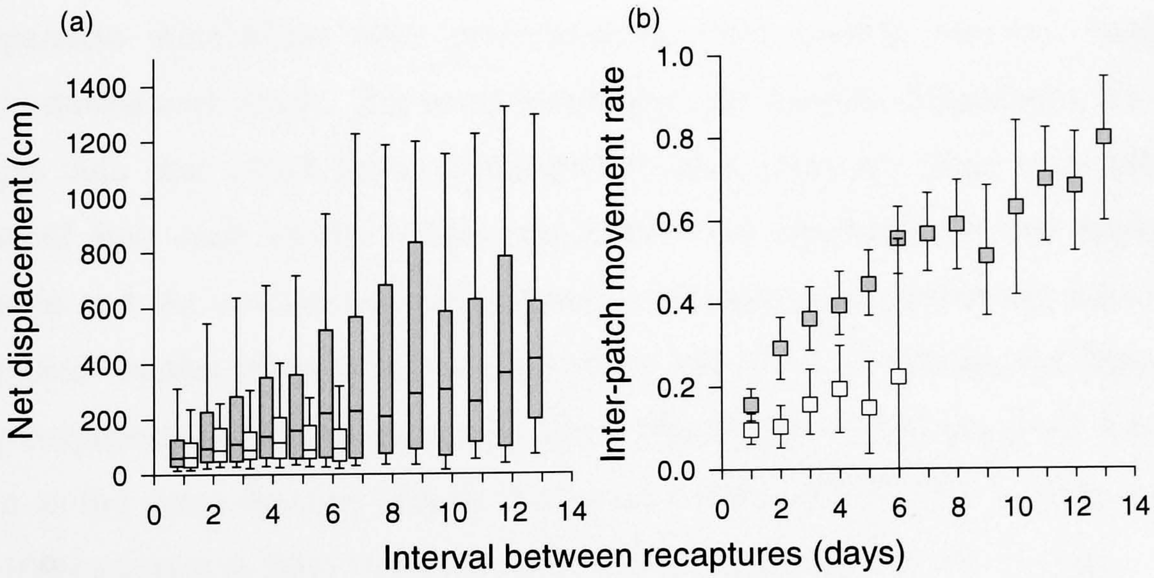


Figure 4.8. (a) Box plots of displacements in the mark-resight studies of 2003 (grey boxes) and 2004 (white boxes). Boxes encompass the interquartile ranges with lines through the medians and error bars delimiting the 10th and 90th percentiles. (b) Inter-patch movement rates (± one standard error) in 2003 (grey squares) and 2004 (white squares).

4.7 Random walk model of movement

4.7.1 Materials and methods

An individual-based, habitat-dependent, correlated random walk simulation model was developed, with the aim of scaling from the behaviour observed in the movement path experiment to the daily displacements observed in the mark-resight studies. This represents a 15-fold extrapolation of spatial scales and a 300-fold extrapolation in temporal scales. The model can be thought of as a coupled map lattice where the real landscape is approximated as a grid of 1 x 1 cm tansy/matrix cells that individuals move over in continuous space. To represent the patch network, elliptical blocks of tansy cells of the correct dimensions are embedded in an infinite matrix, with a reflective boundary along the river edge. Beetles are represented as a pair of Cartesian coordinates (for their location), a bearing (for the direction they are facing) and an internal clock (for the duration of their movement). The simulation was designed to mimic the mark-resight studies and proceeded as shown in Figure 4.9, with movement in each habitat achieved by drawing step lengths and turning angles from the distributions observed in the movement paths.

Independent simulations were performed for each interval between resightings (days) during each study. For every simulation, the number of beetles in the mark-resight data that could have contributed to that interval's data were randomly scattered into each patch. Their movement was simulated for the appropriate duration and the median net displacements and inter-patch movement rates of the 'resighted' beetles (those inside a patch at the end of the simulation, see Figure 4.9) were outputted along with the resight rate. The lengths of the simulated days were taken as the mean daytime lengths during each study period (980 minutes in 2003 and 1000 minutes in 2004) because tansy beetles are almost inactive at night.

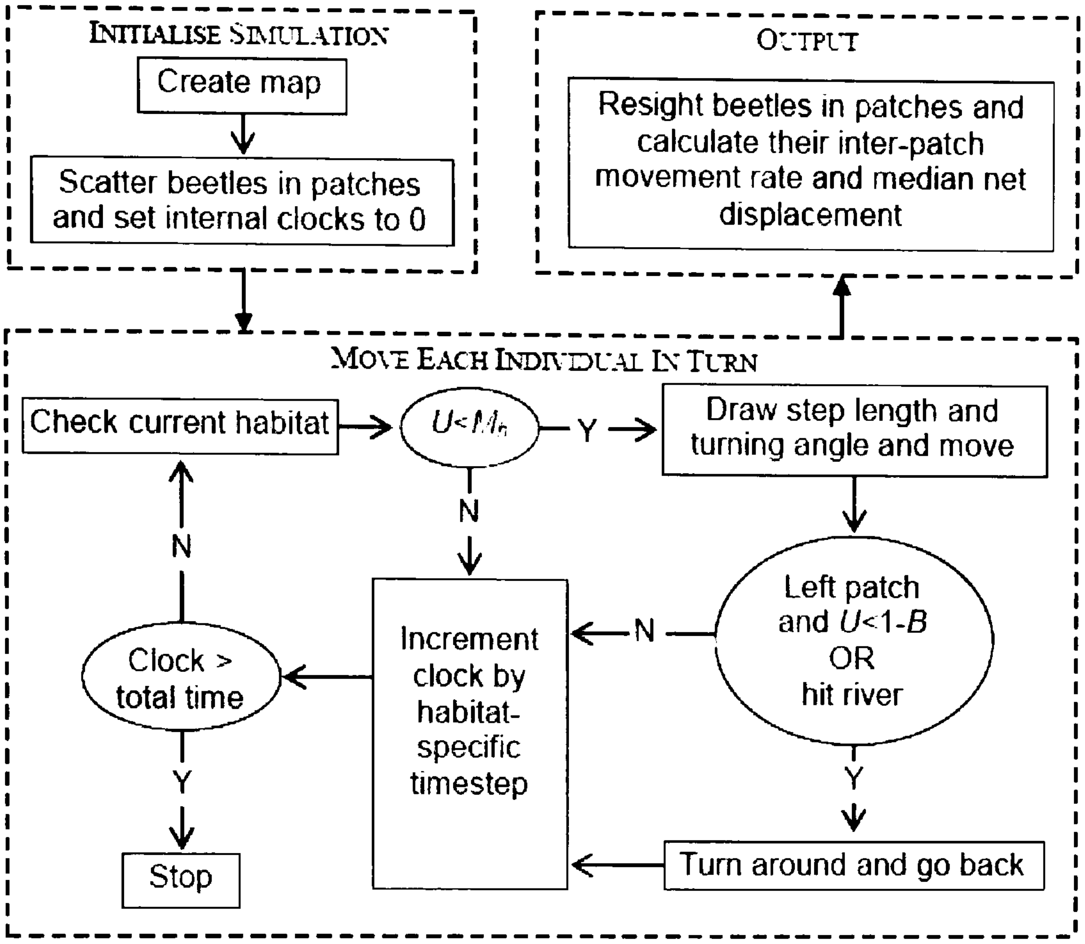


Figure 4.9. Flow chart for the random walk simulation model. After creating the landscape map, beetles are scattered randomly into their starting tansy patches from the real datasets. Each beetle is moved for the duration of the simulation by checking which habitat type it is in and moving accordingly. U is a random deviate drawn from a uniform distribution between 0 and 1, M_h is the observed proportion of time spent moving in habitat h and B is the asymmetric tansy patch boundary permeability (the probability of crossing the boundary when leaving the patch). After unsuccessfully attempting to leave a patch or enter the river, beetles perform U-turns – equivalent to walking back down the stem or leaf that carried them to the boundary. After each movement attempt, beetles' internal clocks are incremented by the duration of movement in that habitat and movement is iterated until the clock time exceeds the simulation length. Once all beetles have been moved, the net displacements and inter-patch movement rates of those that ended inside a tansy patch are outputted – emulating the mark-resight studies. The resight rate is also outputted.

No data on patch boundary permeability exist, but since tansy beetles cannot detect tansy from even short distances (see above), boundary-mediated behaviours are likely to affect emigration but not immigration. Therefore, asymmetric patch-leaving boundary permeabilities, equivalent to the probability of successfully crossing from the patch to the matrix (Figure 4.9), were included. Boundary strengths were varied to explore their effects on the model fit, effectively using the pattern-oriented modelling approach (Wiegand *et al.* 2003) to infer the most likely strengths given the movement model and the observed displacements. Each period between resightings (in days) was simulated independently with boundary permeabilities B between 0 and 1 (0.1 steps). The means of the simulated median net displacements and inter-patch movement rates across all replicates were calculated for all boundary strengths. Their relative error against the field data, Δ, was calculated using the measure of Wiegand *et al.* (1998), $\Delta = [(1/n)\Sigma_d(O_d - E_d)^2]^{0.5}$, where O_d and E_d are the observed and expected (model-predicted) data from the period of length d days and n is the total number of periods with data. The best fitting boundary permeability was selected as that with lowest relative error in median net displacements and inter-patch movement rates. Model-predictions with and without optimal boundary strengths were compared to the observed data by determining whether the observed lay within the 2.5th and 97.5th percentiles of the output from 1000 replicate simulations (the 95% simulation envelope).

4.7.2 Results

The random walk model gave a good match to the observed net displacements of beetles in each plot (details not shown), indicating it to be a minimal adequate model of beetle movements at small scales. The relative errors of the model to the mark-resight data with varying patch boundary permeability are shown in Figure 4.10. For the 2003 data, relatively permeable boundaries ($0.4 \leq B \leq 1$) give the best fits, while for 2004, a strong but not impermeable boundary (B=0.1) was best. When simulated with such boundary values, the model's 95% prediction envelopes corresponded closely with the observed median net displacements and inter-patch movement rates (Figure 4.11). Model-predicted resight (recapture) rates after one day's movement were higher than the field estimates (Table 4.1), which is consistent with failing to find some of the beetles on tansy patches in the field.

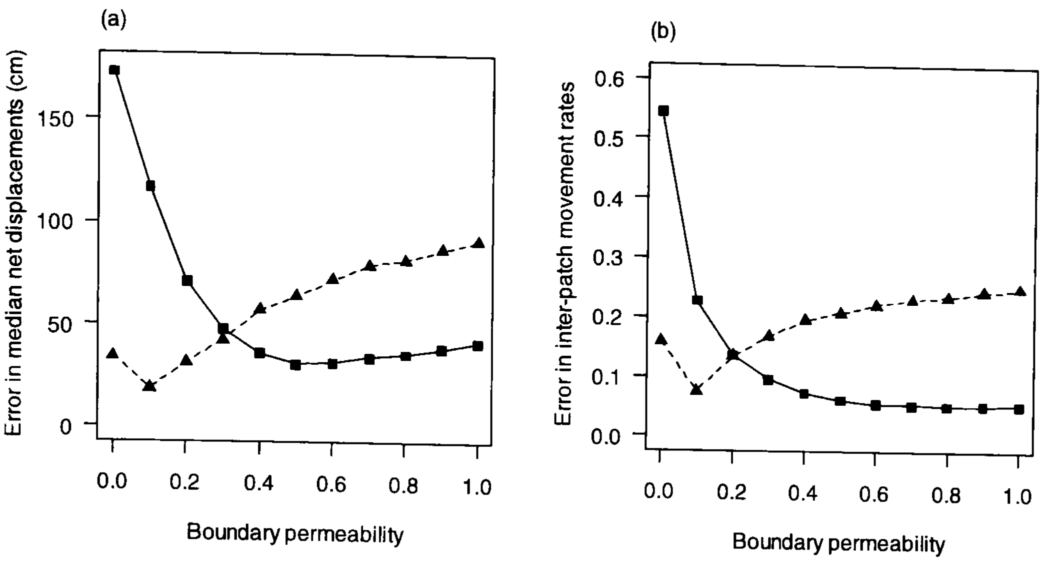


Figure 4.10. Relative errors in the (a) median net displacements and (b) inter-patch movement rates with different tansy patch boundary permeabilities *B*, for simulated movements in 2003 (squares and solid lines) and 2004 (triangles and dashed lines).

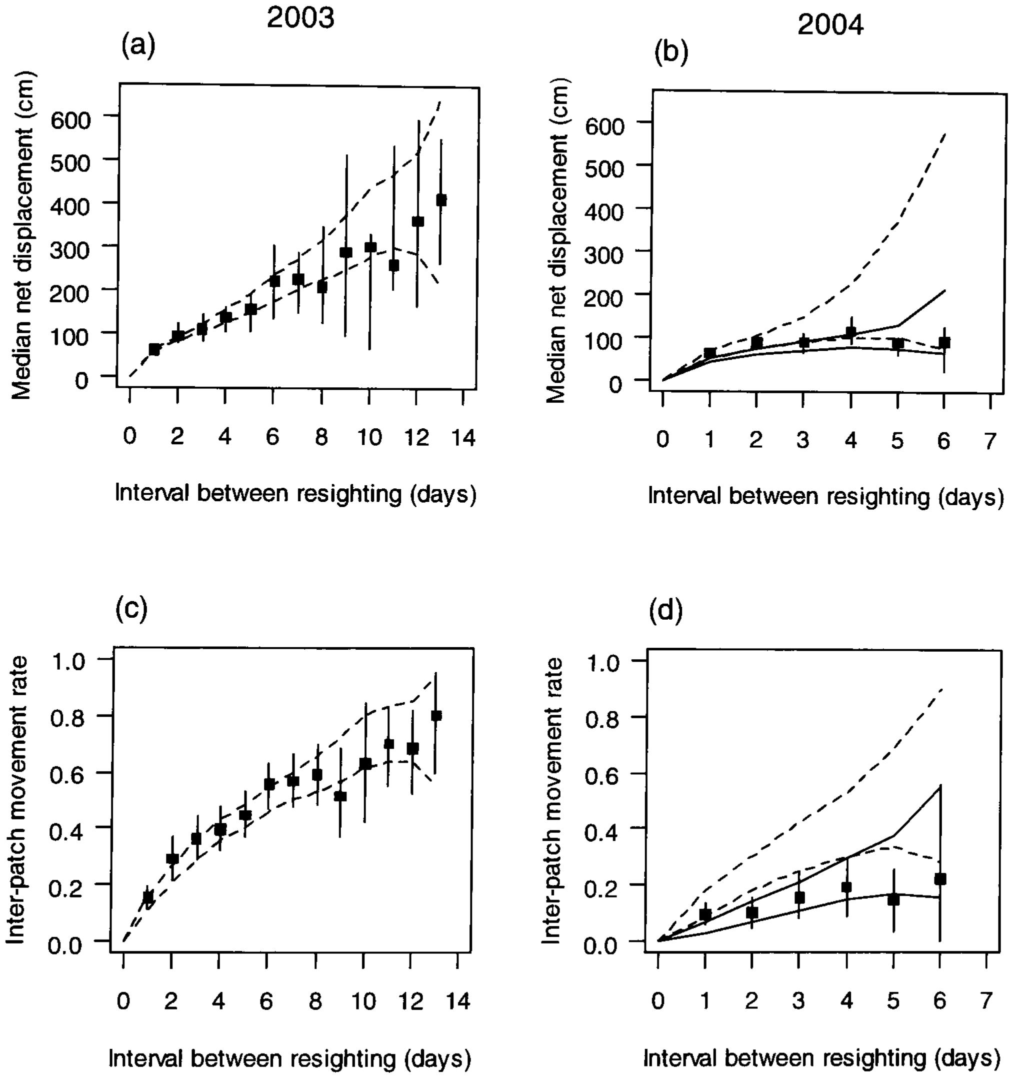


Figure 4.11. (a and b) Median net displacements and (c and d) inter-patch movement rates observed in the mark-resight studies and those produced by the random walk model for 2003 and 2004. Observed data are shown as squares with error bars for their 95% confidence intervals estimated by 1000 bootstraps. Lines show the 2.5th and 97.5th percentiles of 1000 replicate model predictions – analogous to a 95% confidence interval for the prediction. The dashed lines are for the model with fully permeable boundaries (B=1) and the solid lines show the effect of including a strong boundary (B=0.1) in the 2003 simulation.

Table 4.1. Recapture rates that were observed (estimated from the mark-resight data) and predicted by the models (proportion of individuals inside a patch after one day of movement) in both years, with 95% confidence intervals in brackets (2.5th and 97.5th percentiles for the simulated data). Boundary permeabilities in the simulations were B=1 in 2003 and B=0.1 in 2004, which gave good fits to the observed movement data (Figure 4.11). Since detection was perfect in the model and imperfect in the field, the higher results of the model are consistent with the lower field estimates.

Year	Field estimate	Model prediction
2003	0.481 (0.459-0.504)	0.715 (0.690-0.740)
2004	0.497 (0.463-0.532)	0.861 (0.829-0.894)

4.8 Discussion

4.8.1 Ability to orient towards tansy and other beetles

The inference from the work reported here is that adult and larval tansy beetles are not able to detect their host plant or each other at a distance, either by olfaction, sight or a combination of the two. When released between two patches, one of which had adult beetles attached to it, beetles showed no preference for the occupied patch. Beetles failed to respond to the presence of tansy extracts, leaflets and entire patches unless they were in contact with them, in which case they slowed their movement. This resulted in lower emigration rates of larvae from tansy leaf discs than ground elder discs and slower and less frequent movement of adults on tansy than in meadow vegetation. Contact detection of host plants using surface waxes is known from many chrysomelids, including *Cassida stigmatica*, which specialises on tansy (Müller and Hilker 2001) and *Chrysolina brunsvicensis* (Rees 1969). Contact recognition involves chemosensory receptors on the antennae or tarsi and can trigger or arrest feeding and oviposition in insects (Renwick *et al.* 1992; Müller and Hilker 2001; Chapman 2003).

The finding that tansy beetles have no tansy remote-detection abilities is in agreement with work on *C. graminis* by Sivell (2003), who showed that the movement paths of tansy beetles on a lawn were not affected by the location of nearby a tansy plant. Choice tests in an olfactometer have also given similar negative results (Duncan Sivell, personal communication). However, other chrysomelids have well-formed olfactory and visual navigatory systems (Bolter *et al.*

1997; Kalberer *et al.* 2001; Szentesi *et al.* 2002; Boitaeu 2005) so the question then is why is *C. graminis* not able to do what so many other species of chrysomelids can?

One possible answer is that the original *C. graminis* colonists of the Ouse were adapted to feed on other species. *C. graminis* utilises a range of host plants across its global range (Chapter 2) so if it switched to tansy after colonisation occurred the beetle may have yet to evolve the ability to sense it. It is not known whether non-British tansy beetles show any host detection abilities, or whether British tansy beetles retain any ancestral orientation towards former host species. However, the lack of host-detecting powers may facilitate the adaptation of dietary range to local vegetation.

Given a lack of conspecific attraction, mate location by *C. graminis* would appear to be difficult. However, it is possible that beetles will move towards one another at close ranges, as is the case for *Leptinotarsa decemlineata* (Szentesi *et al.* 2002) but were unable to detect individuals fixed to tansy patches that were over a metre away. Thus, laboratory choice tests with individual beetles are required to conclusively demonstrate that beetles cannot detect one another. It would also be interesting to investigate how often contacts between beetles are made using the random walk model, to ask whether or not the model-predicted levels of contact are sufficient to account for observed frequencies of mating recorded in the field. However, beetles in the field may find it easier to find mates than those in the model as their within-patch movement will be somewhat 'channelled' by the arrangement of tansy stems, as is not the case in the model. Moving along stems may cause beetles to accumulate on tansy flower heads at the tops of the patches where it may be possible to randomly locate mates. The hilltopping behaviour of butterflies may have a similar role (Singer and Thomas 1992; Pe'er *et al.* 2006).

4.8.2 Beetle movement behaviour

Beetle movement paths differed when inside and outside tansy patches. Within patches, movements were shorter in length and duration, relatively less common and had wider turning angles. These behaviours lead to lower net displacements in patches and therefore reduce the chance of straying from the patch and entering the unprofitable matrix. Matrix movement paths had more constrained turning angles, rendering them more linear and increasing net displacement. This is a recognised strategy of organisms that suffer high mortality while searching for resources they cannot detect at distance (Zollner and Lima 1999) and is known from other beetles (Wiens *et al.* 1997), other insects (Kindvall 1999; Schooley and Wiens 2003) and also vertebrates (Bowne and White 2004; Phillips *et al.* 2004).

Hunger appeared to increase movement rates in the inter-patch matrix, as beetles starved for 24 or 48 hours reached tansy patches faster than those that had recently fed. However, those starved for 48 hours were slower than those deprived of food for only 24 hours, possibly because their energy reserves were running low. Hunger is known to increase movement rates in *Eleodes* beetles (McIntyre and Wiens 1999a), and as such may lead to long-distance dispersal events. Despite the fact that effects of hunger were not included in the random walk model, it was able to produce realistic patterns of inter-patch movement rates in median net displacements. This may be because most beetles in the model never 'became hungry', i.e. spent longer than 24 hours outside of a tansy patch. Thus, when simulating movements in dense habitat patch networks and for relatively short time periods, hunger may not require consideration in the model, although this is unlikely to be true for longer periods and sparser patch networks where inter-patch movement will take longer.

Movement activity declined to almost zero at night. Since movement tended to decline after nightfall and then pick up after dawn, the reduction in movement was probably caused by changes in temperature rather than light levels. It may be misleading to interpret the pattern of activity during daytime as representative as that in the field however, as the greenhouse effect within the petri dishes meant that lethal temperatures were achieved. This probably increased activity as beetles were moving around to try to escape the heat. Thus, the movement rates observed in the dishes were higher than those seen in the more natural conditions of the movement path experiments. In the random walk model, a simple activity schedule was applied – beetles were active throughout the daytime and inactive at night. This was probably a sufficiently realistic approximation since the activity after nightfall was mirrored by the lag after sunrise.

4.8.3 Extrapolating movement paths to landscape-level movement

There have been few successful attempts to predict or explain landscape-level movements in field settings from the fine-scale movement behaviour of individuals. Those achieving this have often utilised complex, parameter-rich and highly species-specific models (e.g. Revilla *et al.* 2004) or have modelled movement with longer timesteps than those used here (e.g. Kindvall 1996). In this chapter I attempted to link individual movement behaviour of the tansy beetle on timescales of minutes to patterns of population redistribution over 13 days using an extremely simple random walk simulation model. By selecting the strengths of patch boundaries, the model predicted observed temporal trends in median net displacements and inter-patch movement rates from two independent field studies.

Daily patterns of beetle movement were quantified with two multi-patch mark-resight studies. Inter-patch movement was more likely over longer intervals and out of patches with high perimeter-to-area ratios. Relatively large patch perimeters will increase the chance of a beetle reaching the patch edge and emigrating in the course of its general foraging movement (Englund and Hambäck 2004). Movement between tansy patches will then occur when beetles happen upon a novel patch after moving in search of tansy in the matrix. The fact that area did not have an independent effect suggests that patch area does not influence movement behaviour itself, which is consistent with the successful prediction of movement patterns by the random walk model. However, small patches will have higher emigration rates because on average they inherently have higher perimeter-to-area ratios. Effects of host plant size on leaf beetle emigration have previously been explained in terms of perimeter-to-area ratios (Kareiva 1985; Turchin 1986) and this may be a general phenomenon for organisms making exploratory movements within patches.

Sex also had little effect on movement in the field, which is to be expected given the lack of sexual differences in beetle movement paths. Interestingly, mating also had no discernible effect on inter-patch movement rates or net displacements.

Copulations in *C. graminis* may last for over 24 hours, which implies that copulating females moved as freely as those not burdened with carrying a male. This is consistent with the finding that weight did not affect beetle movement paths, although the weight increase experienced by copulating females will be outside the range of individual female weights.

To try to explain these daily movement patterns in terms of behaviour, beetle paths were independently observed within homogeneous plots of tansy and meadow vegetation and a habitat-dependent correlated random walk simulation model of beetle movement was created from these data. Simulations of the model were designed to mimic the mark-resight studies by superimposing movement onto maps of the real patch networks over 13 days in 2003 and six days in 2004, representing approximately 85% and 65% of respective beetle life expectancies at these times. The permeability of tansy patch edges to emigrating beetles was varied and the fits of the model to the central trends of observed population redistribution (median net displacements and inter-patch movement rates) were compared. For the 13-day (2003) study, weak boundary strengths ($0.4 \leq B \leq 1$) were optimal, while strong but not totally impermeable boundaries ($B=0.1$) were optimal for the 6-day (2004) study. Thus, the simplest model was selected as the most plausible for the 13-day study – that without patch boundaries. With optimal boundary strengths, the model's 95% prediction envelopes encompassed the majority of observed median net displacements and inter-patch movement rates, suggesting a good fit to the observed data. Furthermore, the prediction envelope was quite narrow, which increases confidence in its fit.

To interpret these findings, one needs to ask why patch boundaries would be permeable in 2003 but relatively impermeable in 2004? A possible explanation is that the 2004 study was performed around a month later than the 2003 study. A consequence of this was that in 2004 tansy patches were much taller than the surrounding matrix of meadow vegetation, which does not grow as quickly in spring. Beetles reaching the patch edge would therefore often find themselves above the level of the surrounding vegetation and would have no alternative but to turn back into the patch. Consequently, stronger boundaries may be expected in 2004 than 2003, which was what the model suggested. A similar phenomenon has been reported for the Hemipteran *Prokelisia crocea*, where the interface between the host plant patch and a bare mudflat matrix presented an almost total barrier to emigration, whereas a vegetated (and so taller) interface presented no barrier at all (Haynes and Cronin 2006). This study suggests that boundary permeabilities might differ between patches and change over time as different vegetation grows at different rates.

Strong boundaries were probably required to fit the model to the 2004 data because after being reflected back into the patch the individual remained close to the edge and had a high chance of hitting it again soon after. Therefore the boundaries had to be very strong to restrict emigration to the levels observed in the field. In reality, if the boundary effects were driven by a height differential at the patch-matrix interface, edges above the level of the matrix vegetation would be almost impermeable while those below would be almost permeable boundaries. Since beetle heights on the tansy patches will be temporally autocorrelated, incorporating height-dependent boundary permeability into a two-dimensional movement model is challenging. Three-dimensional models, accounting for plant architecture, are a recent development that in the future may be used to address such phenomena (Hannunen 2002).

In modelling movement, it is important to define landscape heterogeneity at the scale at which the organism interacts with it. Here the patch and matrix were treated as uniform environments, although there may have been heterogeneity from the point of view of the beetle, which could have affected movement (Kareiva 1985; Bach 1988; Johnsen *et al.* 200; Revilla *et al.* 2004; Stevens *et al.* 2004; Haynes and Cronin 2006). This would also apply if the altered structure of the plots upon which the movement paths were recorded prevented the model's application to the more natural vegetation in the field. However, since tansy beetles move on dense vegetation with few non-connecting structures, they should have a high degree of control over their movement (Hannunen 2002) so this may be of little importance. Indeed, Goodwin and Fahrig (2002) found that although the goldenrod beetle *Trirhabda borealis* moved differently in different matrix environments, matrix elements only had a small influence on landscape connectivity compared to habitat patches.

Another factor not represented in the simulation model was heterogeneity in beetle movement behaviour. This would increase the proportion of individuals moving either very far or hardly at all, so may have a limited effect on central trends such as those examined here (Skalski and Gilliam 2003). Furthermore, there was only evidence of a weak age-related heterogeneity in the movement path data, which should have been of no importance to the model because beetles in each field study were from the same age cohort. The trend for old beetles to move slightly less often in the matrix may be a further explanation of why the random walk model without boundaries over-predicted inter-patch movement rates in the 2004 mark-resight study, which was performed later in the lifetime of the beetles than the 2003 study.

4.8.4 Conclusions

Tansy beetles seem unable to move towards tansy, either as adults or larvae, although they appear to detect when they are on it and react by decreasing their net displacements. Hunger promotes adult movement and beetles remain largely inactive at night. Beetle movement on tansy was characterised by short steps with wide turning and a short duration of movement. When away from tansy however, beetle movement lasted longer, was over greater distances and followed more linear paths. These patterns were the same for males and females although age apparently reduced the frequency of movement in the matrix.

A minimal model of tansy beetle movement behaviour was able to explain the major elements of its wider-scale, population-level movement (net displacements and inter-patch movement rates), provided that appropriate boundary strengths operate. The field site was limited in size but covered distances for which over 90% of fortnightly net displacements occurred (Chapter 5). For some organisms, random walks might not be able to produce realistic patterns of landscape level movement because they make non-random movements (Conradt *et al.* 2001) or make movement decisions on multiple scales (Nams 2005). However, for *C. graminis*, this does not appear to be the case at a local landscape level. Therefore, using habitat-dependent correlated random walks in spatial ecological models can be appropriate for some species. However, habitat boundary permeabilities will be important aspects of such movement models, and these may vary in time and space depending on their underlying causes.

Chapter 5: Dispersal

5.1 Introduction

Ecology is becoming an increasingly spatial discipline. Some of the most important issues in the field, such as the spread of invasive species, responses to climate change and habitat fragmentation, are inherently spatial and as such, dispersal is now viewed as a fundamental process affecting individual fitness, population dynamics, species' distributions, community structure and evolution (Clobert *et al.* 2004; Bowler and Benton 2005; Cottenie 2005). Realistic models of dispersal are therefore required to address many key areas of ecological research.

Although highly desirable, it is often difficult to develop easily-parameterised models of movement behaviour at small scales, such as random walks, that generate realistic distributions of dispersal distances (dispersal kernels). This is partly because such models predict Gaussian kernels (Turchin 1998; Okubo and Levin 2002) while measured ones are typically leptokurtic, with 'fatter' tails of long-distance dispersal (Kot *et al.* 1996). This can be caused by heterogeneity in movement behaviour (Skalski and Gilliam 2003) or by responses to specific landscape features such as habitat-patch boundaries (Morales 2002), which may be hard to quantify accurately and incorporate into models of movement at large spatial scales.

An alternative approach is to measure dispersal and then fit general functional forms that characterise the kernel to the data (Turchin 1998). An example of this is the Virtual Migration Model for movement between habitat patches within metapopulations (Hanski *et al.* 2000), which uses mark-recapture data to parameterise a dispersal model with a negative exponential kernel. However, the negative exponential may give a worse fit to empirical data than alternative forms, both in plants (Skarpaas *et al.* 2004) and animals (Hill *et al.* 1996; Kot *et al.* 1996; Thomas and Hanski 1997; Baguette 2003). The precise form chosen for the dispersal function can affect the outcome of dispersal models, so it is important to choose a function that is appropriate to the species under consideration (Kot *et al.* 1996).

There are many potential proximate drivers of dispersal, which also require consideration in dispersal models (Clobert *et al.* 2003; Bowler and Benton 2005). For example, individuals may be more likely to emigrate from patches with high population densities because of resource depletion or conspecific interactions, as is the case for the chrysomelid beetle *Trirhabda virgata* (Herzig 1995). Patch size may also play a role, whereby emigration is typically high and immigration low in small patches (Kareiva 1985; Turchin 1986; Hill *et al.* 1996). High emigration from small patches may be caused by increased encounters with the patch edge (Englund and Hambäck 2004) while reduced immigration may result from a lower chance of finding a small patch (Byers 1986) or a reduced chance of the disperser choosing to settle into a small patch with a small population. There may also be sex-differences in dispersal, as females aim to locate multiple oviposition sites, while males act to gain access to females. For example, female *Parpxyna plantaginis* flies exhibit density-dependent dispersal, probably selected by competition for flower heads to lay eggs on, while males sit and wait for females, rendering their dispersal independent of density (Albrechtson and Nachman 2001).

The aim of this chapter is to investigate and model dispersal of *Chrysolina graminis* moving between patches of its host-plant tansy. Beetle dispersal was recorded within large patch networks over two years with mark-recapture and the effects of time, patch area, sex, flooding and beetle density on the likelihood and magnitude of dispersal were analysed. In order to contrast different dispersal kernels, I develop a general model for the dispersal of insect herbivores between host-plant patches, fit the model to the mark-recapture data with each kernel and compare their performance.

5.2 Observed dispersal

5.2.1 Materials and methods

Data on dispersal were collected in a multi-patch mark-recapture study performed in 2004 and 2005 on roughly 2 km of riverbank at Clifton Ings, just north of York city centre (Figure 5.1). Here, tansy grows along the unmanaged riverbank and on an annually mown flood embankment that bounds the bank. Downstream, the bank is wooded and contains no tansy. Upstream, conditions are similar to Clifton, so only tansy patches in the downstream half of the site were included in the study to minimise the influence of upstream patches.

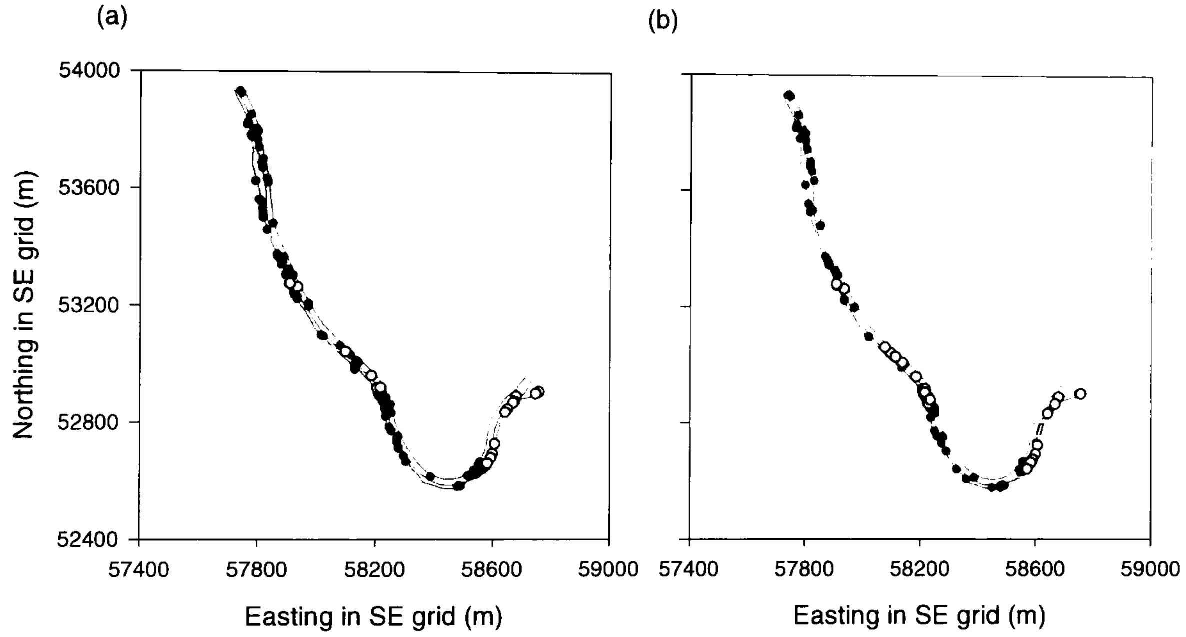


Figure 5.1. Maps of the tansy patch networks at Clifton Ings in (a) 2004 and (b) 2005. The river runs below and to the left of the three lines, which show the boundaries of the riverbank. The middle of the three lines divides the bank (nearest to the river) and the flood embankment. Patches of tansy are shown as dots, the grey ones being the patches visited as part of the mark-recapture studies.

At the start of each field season, the site was thoroughly searched for tansy and patches were defined as tansy clumps less than 0.5 m apart. Their locations were mapped with a hand-held GPS (Garmin GPS 12) and their basal lengths and widths measured with a tape measure. Basal areas were estimated as those of ellipses with these dimensions. In 2004, 158 tansy patches were found while there were 126 in 2005. Twenty-five of these were selected for study in 2004 and 32 were chosen in

2005. In general, clusters of patches containing high numbers of beetles were selected as it was thought that the majority of dispersal events would be over short distances. In 2004, beetles found on all visited patches were marked, while in 2005 this was the case for 24 of the 32 study patches. The remaining eight patches were monitored for the immigration of previously marked beetles but no marking was carried out on them, allowing a greater number of patches to be visited.

Beetles were permanently marked by lightly abrading the surface of their elytra using a cordless engraving drill (Dremel Multi 7,2V) to leave an individual code of dark dots (Figure 5.2). Each elytron was divided into three horizontal rows (top, middle and bottom) and each row was divided into three marking positions (inner, central and outer). At each row, beetles were either given a single dot at one marking position or the row was left clear, resulting in 4095 unique permutations (excluding all rows unmarked). A similar marking system has previously been used for the species and is not thought to cause lasting injury (Sivell 2003).

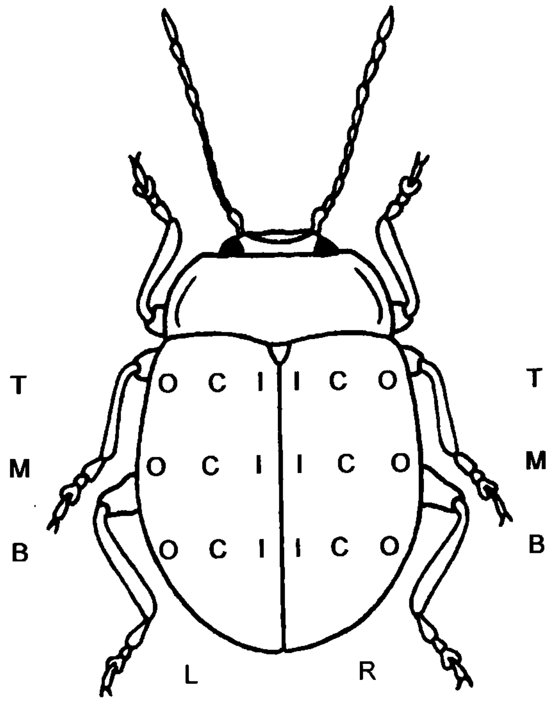


Figure 5.2. Diagram of the drill-marking positions for tansy beetles in the mark-recapture study. The left and right elytra (L and R) were divided into three rows – top middle and bottom (T, M and B). Each row was either left blank or given a single dot at its inner, central or outer positions (I, C or O). Thus beetles had a maximum of six drill-marks and there were 4095 unique marking code.

The study patches were visited every 14 days (±1-2 days on three occasions, two of which were as a result of flooding) from 21st April to 6th October 2004 and 12th April to 11th October 2005, encompassing almost all of the species' active period. Two weeks is approximately half the life expectancy of an active beetle (Sivell 2003) and since beetles must feed over this period, all successful dispersal events are likely to be within this time. During each visit beetles were collected by hand, unmarked beetles were sexed and marked (if on an appropriate patch) and the identities of all recaptured beetles recorded. Beetles were then released back into the centre of the patch in which they were found. Following this, the areas of the study patches were measured in the same way as in the surveys to monitor patch growth and to calculate relative beetle density from the number of adults sampled. Patch coordinates were also re-recorded so that error in the GPS readings was minimised by taking their means throughout the seasons.

Beetle capture histories were processed by extracting pairs of successive captures (with no intervening captures) of the same beetle in the same year and recording whether movement between patches had occurred. If so, the beetle's net displacement was calculated as the straight-line distance between the centres of the two patches. For simplicity, analyses of dispersal rates and distances considered the intervals between visits to be whole fortnights and different pairs of recaptures from the same beetle to be independent.

5.2.2 Results

5.2.2.1 Summary of the data

Overall, 2226 beetles were marked in the course of the study, of which 1622 were captured in 2004 and 794 were captured in 2005 (Figure 5.3a). Marked beetles were recaptured an average of 0.69 times each. The capture histories contained 1032 non-overlapping pairs of successive recaptures in 2004 and 304 in 2005. Dispersal events (inter-patch movements) comprised 36.3% and 33.2% of these data in 2004 and 2005, respectively, over intervals from one to 10 fortnights. There was an apparent peak in dispersal prior to the time of adult emergence in mid-summer in both years (Figure 5.3b) although this was when the embankment patches had been mown and during a time of flooding, which might have been responsible for the peak. When dispersal occurred, fortnightly median net displacements were 8.84 m in 2004 and 9.56 m in 2005, although the maximums were 460.6 m in 2004 and 456.0 m in 2005 indicating that a minority of the data comprised long-distance dispersal events.

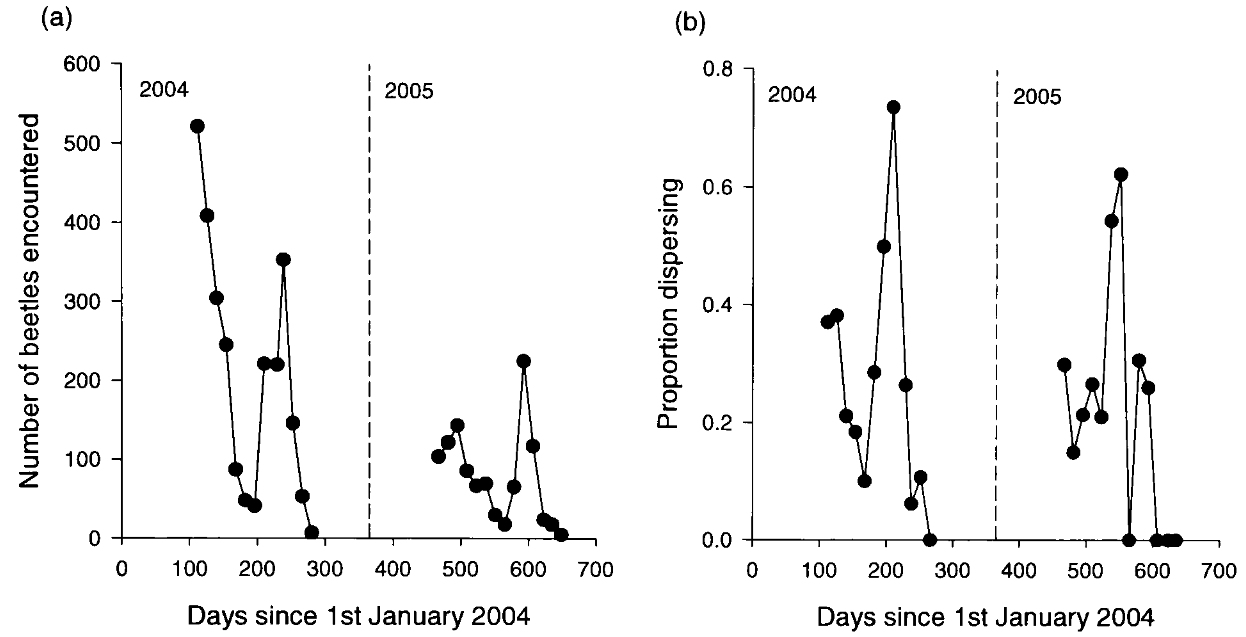


Figure 5.3. (a) The number of beetles caught in each visit of the mark-recapture study. Assuming roughly constant detection probabilities, a general decline in the beetle population is evident. The emergence of the new generation of adult beetles resulted in the spike midway through each season. (b) The mean proportion of individuals dispersing (moving between tansy patches) through time at Clifton Ings between each visit and the next one (excluding winter). There is an apparent peak in dispersal prior to adult emergence.

The reason for the apparent population decline (Figure 5.3a) may have been that the population was extremely high at the start of 2004 leading to many of the well-populated tansy patches being over-exploited and degraded by high numbers of beetle larvae. This meant that fewer adult beetles emerged in the summer of 2004 so the population was lower in 2005. Additionally, there was severe flooding just after adult emergence in the summer of 2004, which may have killed many of the new generation of adults. In 2005, the tansy patches that were highly degraded in 2004 tended to grow poorly and supported fewer beetles. Also, no new adult beetles emerged in any tansy patches on the flood embankment in 2005, which was not the case in 2004. The flood embankment is mown annually in the summer, causing a temporary absence of available tansy foliage from this part of the site. Variability in the precise timing of the beetle's lifecycle may have meant that mowing in 2005 occurred before larvae were ready to enter the underground pupal stage and so caused the direct mortality or starvation of the embankment subpopulations.

5.2.2.2 The 'decision' to disperse

When dispersal (inter-patch movement) occurred, beetles moved up and downstream in equal numbers (N=476, χ^2_1=1.016, P=0.313). Forward stepwise logistic regression on the binary variable of whether or not dispersal occurred showed that it was more common over longer intervals (Figure 5.4), if the beetle was female, originated in a smaller tansy patch and if the interval between recaptures included a period of flooding (of which there were two in 2004 and none in 2005) (Table 5.1). There were no significant differences between years and adult beetle density did not affect dispersal rate. As well as increasing dispersal rates, flooding caused greater than expected movement from the riverbank patches onto the flood embankment (Table 5.2).

Table 5.1. Forward stepwise logistic regression results for the probability of moving between patches at Clifton Ings (N=1336). Positive regression coefficients indicate an increase in the probability of dispersal with an increase in that variable. The year of the study was omitted from the regression model (P=0.213). Although highly significant, the regression explained only a small part of variation in the data ($R^2_{\mathrm{Nagelkerke}}$=0.093).

Variable	**Coefficient (SE)**	**Wald$_1$**	***P***
Constant	-0.720 (0.160)	20.33	<0.001
Interval between recaptures (fortnights)	0.384 (0.056)	46.82	<0.001
Starting patch basal area (m^2)	-0.181 (0.050)	13.19	<0.001
Sex (coded males=1, females=0)	-0.365 (0.119)	9.387	0.002
Flooding	0.510 (0.184)	7.661	0.006

Table 5.2. The effects of flooding on the direction of movement. Table cells show the observed frequencies of movements over fortnightly intervals in 2004 between the riverbank and the flood embankment when flooding did and did not occur and those expected if flooding had no effect on movement (in brackets). There was no flooding in 2005 so these data were excluded. During times of flooding, movement away from the riverbank was significantly more common than expected while movement onto the riverbank was significantly less common (N=145, χ^2_1=8.908, P=0.003).

	Bank to embankment	**Embankment to bank**
Flooding	26 (19.2)	3 (9.8)
No flooding	70 (76.8)	46 (39.2)

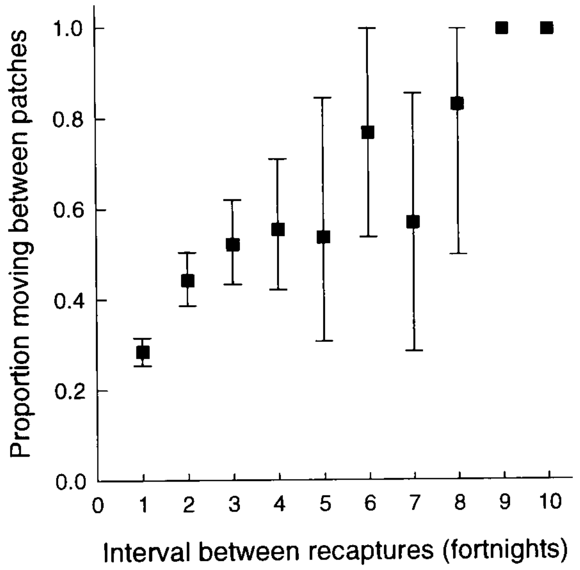


Figure 5.4. Mean proportion of individuals dispersing (moving between tansy patches) over different intervals, with 95% confidence intervals estimated through 1000 bootstrappings. After 9 fortnights within the same season all surviving individuals had dispersed.

5.2.2.3 Dispersal distance

Net displacements during dispersal (centre-to-centre distances between patches) were not normally distributed (Kolmogorov-Smirnov test, N=476, Z=7.334, P<0.001), being highly leptokurtic (kurtosis of 21.17) and right-skewed (skewness of 4.228) (Figure 5.5). They could not be normalised with standard transformations so the effects of year, sex and interval between recaptures on dispersal distance were assessed with a Scheirer-Ray-Hare (SRH) test, the non-parametric equivalent of multi-way ANOVA (Sokal and Rohlf 1995). Neither year (H_1=0.241, P=0.624), sex (H_1=0.151, P=0.698), nor interval (H_9=2.664, P=0.976) affected dispersal distance and there were no significant two- or three-way interaction terms (all P>0.6).

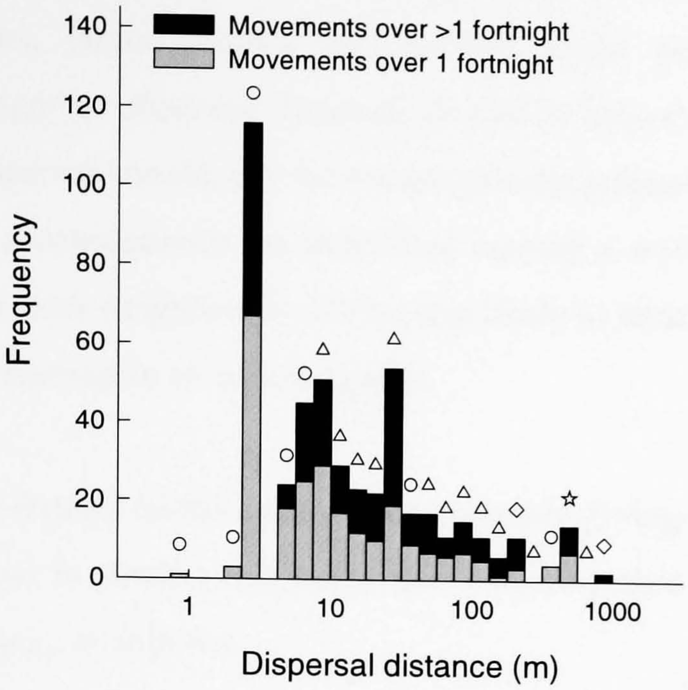


Figure 5.5. The net displacements of dispersing beetles (those moving between tansy patches) (N=446) in the mark-recapture study over one fortnight and longer periods. Note the log-scale on the *x*-axis. Symbols above a bar indicate that there were pairs of study patches within that distance class (and hence the possibility of observing dispersal), with the number of pairs denoted by the symbol (circles = 1-5, triangles = 6-20, diamonds = 21-100, stars =>100).

5.3 Modelling dispersal

5.3.1 Material and methods

5.3.1.1 A model for the dispersal of herbivorous insects between host-plant patches

A simple model of the dispersal of insect herbivores was developed and fitted to the mark-recapture data. I assume that individuals on host-plants continually make small exploratory movements, which means that there is a temporal scale above which all individuals in even the largest patches are likely to leave their patch. Based on the random walk model of beetle movement developed in Chapter 4, this is likely to be the case over two weeks, which is the smallest resolution of the mark-recapture data. Thus emigration did not require explicit consideration in the model.

Once an individual has wandered out of its original patch it will move around in the inter-patch matrix in search of host-plants and will not discriminate between the patch it has left and a novel one. Even without specific adaptations for orientation towards host-plants, larger patches will present larger targets and will retain immigrants for longer so observed dispersal should be biased towards large patches (Byers 1996). Dispersal should also be biased towards patches that are closer to the original host. As a consequence, an individual leaving a well-connected patch (i.e. one with large and close neighbours) will be less likely to return to the original patch than an individual starting in an isolated patch.

These patterns are framed mathematically in a formula giving the probability that at time *t*, an individual in patch *j* will move into patch *k* rather than any of the other available patches, $\psi_{jk,t}$, as follows,

$$\psi_{jk,t} = \frac{f(d_{jk})A_{k,t}^{\ b}}{\sum_{l=1}^{Q} f(d_{jl})A_{l,t}^{\ b}} \qquad \text{(Equation 5.1)}$$

where Q is the total number of patches at the site, $A_{k,t}$ is the area of patch *k* at time *t* and $f(d_{jk})$ is a function of the distance between *j* and *k*, d_{jk}, which describes the dispersal ability of the species (the dispersal kernel). The parameter $b > 0$ scales the relative bias in movement to large patches. Five alternative forms of $f(d_{jk})$ are shown in Table 5.6, all of which give the proportion of individuals capable of moving d_{jk} and can take any form that gives $f = 1$ for $d = 0$ (since all individuals can move 0 m) and then declines towards zero for greater distances. Different kernels give different shaped curves so it is important to select the most appropriate for describing the movement capacity of the focal species.

The model works by giving the movement from *j* to *k* a weighting based on the ability of the species to cover the distance between the patches and the area of *k*. The realised probability of movement between the two is therefore their weighting divided by the sum of the weightings for all the patches at the site (the denominator in Equation 5.1). Landscape structure (the distribution of patches) is thus accounted for in determining movement probabilities. The Virtual Migration Model (Hanski *et al.* 2000) and the patch accessibility model of Heinz *et al.* (2005) use similar equations whereby patches 'compete' for dispersers on the basis of their size and distance from the source, but here all individuals rather than those that have 'decided' to disperse are competed for and the source patch participates in the competition, resulting in a simpler model. Note that small patches will achieve higher emigration rates as they have weaker competitive abilities.

Table 5.3. Alternative dispersal kernels $f(d_{jk})$, whose performance in explaining the tansy beetle data were compared. All models give the proportion of individuals able to move as far as the distance between patches j and k, d_{jk} in terms of parameters $\sigma > 0$, $\alpha_i > 0$ and $\beta_i > 0$. G is a Gaussian kernel, with a mean of 0 and a variance of σ, normalised so that $f(0) = 1$, as would be the expectation of a simple diffusion model (Turchin 1998; Okubo and Levin 2002). The negative exponential (NE) is frequently used in metapopulation models (Hanski *et al.* 2000; Moilanen 2004) and is attractive because $1/\alpha_1$ gives the mean dispersal distance. The other kernels have longer tails (i.e. increased long-distance dispersal). The inverse power function (IP) is commonly fitted to empirically measured distributions of dispersal distances (Hill *et al.* 1996). The extended negative exponential (ENE) is similar to NE but has an extended tail and is of the form used by Taylor (1978). G and NE are both special cases of ENE. The fat-tailed kernel (FT) is used in the incidence function metapopulation model (Moilanen 2004).

Kernel	**Abbreviation**	$f(d_{jk})$
Gaussian	G	$\exp\left(\frac{-d_{jk}^2}{2\sigma^2}\right)$
Negative exponential	NE	$\exp(-\alpha_1 d_{jk})$
Inverse power function	IP	$\begin{bmatrix} 1 & \text{if } j = k \\ \min(1, d_{jk}^{-\alpha_2}) & \text{if } j \neq k \end{bmatrix}$
Extended negative exponential	ENE	$\exp(-\alpha_3 d_{jk}^{\beta_1})$
Fat-tailed kernel	FT	$\frac{1}{1+\alpha_4 d_{jk}^{\beta_2}}$

If patch areas were larger or the timestep smaller, so that all individuals did not leave their patch between captures, the model could be extended to cover emigration as follows,

$$\psi_{jk,t} = \begin{bmatrix} \eta A_{j,t}^{-c} \dfrac{f(d_{jk}) A_{k,t}{}^{b}}{\sum_{l=1}^{Q} f(d_{jl}) A_{l,t}{}^{b}} & \text{if } j \neq k \\ \left(1 - \eta A_{j,t}^{-c}\right) + \eta A_{j,t}^{-c} \dfrac{f(d_{jk}) A_{k,t}{}^{b}}{\sum_{l=1}^{Q} f(d_{jl}) A_{l,t}{}^{b}} & \text{if } j = k \end{bmatrix} \qquad \text{(Equation 5.2)}$$

where parameters $\eta > 0$ and $c > 0$ scale emigration rates from the source patch j to its area (Hanski *et al.* 2000; Englund and Hambäck 2004). If the source and target patches are different, $\psi_{jk,t}$ is the probability of emigrating from j multiplied by that of moving to k. Where they are the same, $\psi_{jk,t}$ is the probability of not emigrating plus that of emigrating then moving back into j.

5.3.1.2 Parameter estimation from observed movements

A maximum likelihood approach was taken to fit the model in Equation 5.1 to the tansy beetle data with each dispersal kernel from Table 5.1 (preliminary investigation with the model in Equation 5.3 suggested full emigration, so the simpler model was preferred). This involved calculating the log-likelihood of every piece of data given the model and some set of parameters then finding the maximum likelihood parameter estimates (MLEs) – the set of model parameters that maximise the log-likelihood of the entire dataset. It is possible to calculate the likelihood of the dispersal recorded within each capture history from the first to the last capture as the product of the likelihoods of all the possible paths from the initial to the final patch, via every combination of patches that the individual could have been in when not captured. However, given the number of patches and duration of this study, the computational power required to evaluate the extremely large number of potential paths is unfeasibly high. Therefore, ‘capture history fragments’ of successive captures over one and two fortnights were extracted from the data. Given some parameters, the log-likelihood of capture history fragment x, $l(x)$ where a beetle started in patch j and ended up in patch k is,

$$l(x) = \begin{bmatrix} \ln(\psi_{jk,t}) & \text{if } x = \{j,k\} \\ \ln\left(\sum_{i=1}^{Q} \psi_{ji,t}\psi_{ik,t+1}\left[1-p_i\right]\right) & \text{if } x = \{j,0,k\} \end{bmatrix} \qquad \text{(Equation 5.3)}$$

where p_i is the capture probability in the intermediate patch i where the beetle was not captured. In the capture history fragment, j and k represent known locations of the beetle and 0 represents a non-capture. If the fragment includes a miss, $l(x)$ is the sum of the log-likelihoods of all possible routes between *j* and *k*, accounting for the fact that the beetle was looked for but not found on one occasion.

Capture probabilities in the study patches were not themselves of interest but had to be estimated for the analysis. A complicating factor is that *C. graminis* goes through periods of underground diapause, both for overwintering and occasionally during the summer. For example, some adults enter the soil after mating in May or June and then reappear in late summer or the following spring, while others delay their emergence from overwintering or hibernate early. Since underground individuals are unobservable, this violates a basic assumption of standard mark-recapture analyses – that of equal catchability. Such a problem can be tackled by including an unobservable state in a multi-state mark-recapture model, but this is complex and parameter-rich. Therefore, I made simple capture probability estimates as the proportion of individuals captured both immediately before and after each visit that were captured at the visit. This assumes constancy across time and patches, but since individuals are unlikely to enter and emerge from diapause in the space of four weeks, the procedure was probably based on active individuals only. Capture probabilities in the unvisited patches were zero. Since capture probability is related to movement through the time spent inside patches, separate estimates were made from each dataset that the model was being fitted to (e.g. male and female capture probabilities were estimated separately when the model was fitted separately but not otherwise). Although simplistic, this method may perform as reliably as more complex alternatives (Hanski *et al.* 2000).

The overall log-likelihood of the dataset, *L*, given some set of parameters, is the sum of the log-likelihoods of individual capture history fragments (Edwards 1992). The maximum log-likelihood, L^* and its MLE parameter values were found by numerical optimisation through 2500 iterations of simulated annealing (Kirkpatrick *et al.* 1983), repeated three times with random seeds to check for convergence.

For the purpose of parameter estimation, patch areas during the interval between visits *t* and *t*+1 were taken as the mean of their areas at *t* and *t*+1. Because it was necessary to know the areas of every patch throughout the field season to fit the model, the areas of the unmarked patches were estimated from their sizes at the start of the season and the mean growth rates of the marked patches thereafter. Error introduced by this should have had limited effect since most of the unmarked patches were far from the clusters of marked ones. For intervals in which flooding occurred, the areas of the bankside patches were halved as the tansy available to the beetles during these intervals was lower than that measured.

The model was fitted for each alternative dispersal function (Table 5.3), and the best fitting of these was selected from their Akaike Information Criteria (AIC_c) (Burnham and Anderson 2002). Using the optimal kernel, models were fitted for the entire dataset, with dispersal being (1) the same for both sexes and years, (2) different between years but not sexes, (3) different between sexes but not years or (4) different between both sexes and years. The AIC_c values of these scenarios were compared to find the best overall model of tansy beetle dispersal.

5.3.1.3 A test of the estimation method

To test the method of parameter estimation, simulated datasets were created and used to obtain maximum likelihood parameter estimates with 2500 iterations of simulated annealing. Data were simulated by randomly placing 500 individuals within a network of just the 25 patches from the 2004 study. The individuals then made a movement according to the assumptions of the model (Equation 5.1) with one of the dispersal kernels (Table 5.3) and arbitrary parameter values chosen from a uniform distribution between 0 and 2. Parameter combinations were only accepted if more than 20% of the individuals moved between patches since it would not be possible to estimate dispersal parameters from a simulated dataset with very few dispersal events. However, a threshold of 10% was used for the Gaussian kernel as few parameter combinations resulted in sufficient numbers of movements. Simulated movements lasted one fortnight with a frequency of 0.771 and two fortnights with a frequency of 0.229, which were the frequencies in the 2004 data. This was repeated 20 times for each kernel and the ‘true’ and estimated parameter values were compared.

5.3.1.4 Estimation of parameter support intervals

The 95% confidence intervals of an MLE may be estimated using likelihood ratio test (Edwards 1992). This involves changing the value of the focal parameter and re-estimating the other parameters with the focal parameter fixed at its new value. The 95% confidence limits are the minimum and maximum parameter values where twice the absolute difference between the new and optimal log-likelihood is less than the critical value of χ^2 with one degree of freedom at α=0.05.

However, it was impractical to use this method for the tansy beetle data due to its computational demands. As an alternative, 2-unit support intervals were calculated around the MLE of each parameter, as the range of parameter space where the log-likelihood exceeded L^*-2 when the other parameters were held at their MLE values. It is thought that 2-unit support intervals are generally of similar, although often slightly lower, magnitudes than true 95% confidence intervals (Edwards 1992).

5.3.1.5 Assessment of model fit

To assess the degree to which this model explained patterns in the data, the observed and expected numbers of emigrants and immigrants associated with each patch were compared. Observed and expected numbers of dispersers in the data throughout the study were also compared to see if there were any phenological patterns in beetle dispersal that were not accounted for by the model. Comparisons were not made using chi-squared tests since many pairs were based on small sample sizes, which can lead to spurious significance, and the observations were not totally independent. Therefore, Pearson's product moment correlation coefficients between observed and expected values were calculated (excluding cases with no data). Significances were assessed by calculating correlation coefficients for 1000 randomisations of the expected values and determining whether the true correlation coefficient lay outside the 2.5th or 97.5th percentile of these.

The expected number of emigrants from patch j over the T visits in the season is

$$\varepsilon_j = \sum_{t=1}^{T-1} N_{j,t}\left(1 - \psi_{jj,t}\right) \qquad \text{(Equation 5.4)}$$

where $N_{j,t}$ is the number of individuals caught in j at visit t who were caught again in any patch at t+1. The expected number of immigrants to j, is

$$\zeta_j = \sum_{t=1}^{T-1} \sum_{\substack{k=1 \\ k \neq j}}^{Q} N_{k,t}\psi_{kj,t} \qquad \text{(Equation 5.5)}$$

The expected number of dispersers in the data between t and t+1, μ_t is

$$\mu_t = \sum_{j=1}^{Q} N_{j,t}\left(1 - \psi_{jj,t}\right) \qquad \text{(Equation 5.6)}$$

5.3.2 Results

5.3.2.1 Testing the parameter estimation

The maximum likelihood parameter estimates (MLEs) derived from simulated data are plotted against the parameter values with which they were simulated in Figure 5.6. The estimation procedure provided unbiased and accurate MLEs (Figure 5.6). In 79 of the 80 cases, the MLEs achieved higher overall likelihoods than the 'true' parameter values showing that the estimation procedure found the best fitting sets of parameters and that the scatter around the lines of equivalence was due to stochastic variation in the data rather than inefficient or inaccurate estimation.

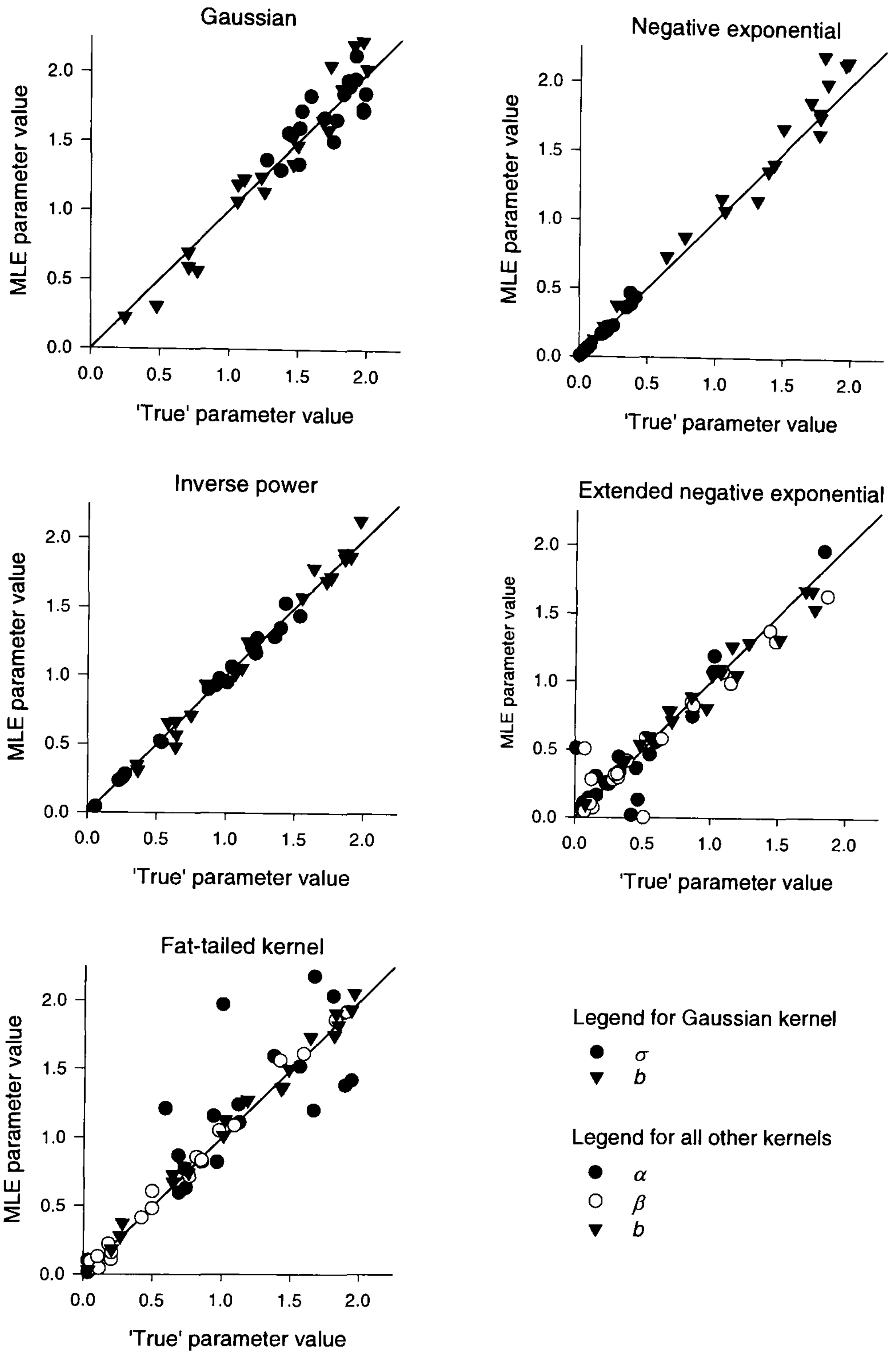


Figure 5.6. Maximum likelihood estimates (MLEs) of the dispersal model parameter values obtained with 2500 iterations of simulated annealing on simulated datasets (N=500) plotted against the 'true' parameter values from which the data were simulated. Twenty replicates for each dispersal kernel in Table 5.3 are shown, as are lines of equivalence between true and estimated parameters.

5.3.2.2 Selection of the best model for tansy beetle dispersal

When the model in Equation 5.1 was fitted to the data for each sex and year separately, there was almost overwhelming support for the fat-tailed dispersal kernel, with the exception of males in 2004 for whom there was also some support for the inverse power function (Table 5.4). The Gaussian kernel performed worst overall, with the negative exponential also giving a relatively poor fit. The extended negative exponential and the inverse power function generally performed similarly.

Table 5.4. The selection of dispersal kernel based on the log-likelihood of their MLEs, L. Each model's Akaike Information criterion (AIC_c) was calculated based on L and its number of parameters k (which includes kernel parameters and capture probabilities). ΔAIC_c (the difference between each model's AIC_c and the minimum in that candidate set) values were calculated. Lower values indicate better support for that model, with values over 2.5 indicating very low support. Akaike weights, w, were also calculated. These are interpreted as the probability that each model is the best of the candidate set. For all datasets, the fat-tailed dispersal kernel performed the best, although there was some support for the inverse power function among males in 2004.

Data	Kernel	L^*	k	AIC_c	ΔAIC_c	w
2004 females (N=454)	G	-1196.3	3	2398.6	892.5	<0.001
	NE	-919.9	3	1845.9	339.8	<0.001
	IP	-763.3	3	1532.7	26.56	<0.001
	ENE	-757.6	4	1523.3	17.23	<0.001
	FT	-749.0	4	1506.1	0	>0.999
2004 males (N=427)	G	-1110.5	3	2227.0	1129.3	<0.001
	NE	-798.7	3	1603.4	505.7	<0.001
	IP	-546.3	3	1098.7	1.001	0.377
	ENE	-555.9	4	1120.0	22.25	<0.001
	FT	-544.8	4	1097.7	0	0.623
2005 females (N=145)	G	-380.0	3	766.2	394.8	<0.001
	NE	-249.7	3	505.6	134.3	<0.001
	IP	-185.9	3	378.0	6.642	0.034
	ENE	-184.8	4	377.9	6.543	0.035
	FT	-181.5	4	371.3	0	0.931
2005 males (N=110)	G	-282.4	3	570.9	298.7	<0.001
	NE	-172.7	3	351.6	79.36	<0.001
	IP	-138.5	3	283.2	10.94	0.004
	ENE	-137.4	4	283.1	10.84	0.004
	FT	-131.9	4	272.3	0	0.991

Fitting various dispersal scenarios to the fat-tailed kernel, I conclude that dispersal ability did not change over the two years of study but differed between sexes, although this is not definitive (Table 5.5). Parameter estimates (Figure 5.7a) suggested that the kernels of males and females were very similar, with females being slightly more mobile. However, the small difference between male and female kernels may not have been significant, which could be the reason for the uncertainty in the previous conclusion. All beetles were unlikely to disperse further than 100 m in two weeks (Figure 5.7b) and had strong bias towards larger patches, particularly in females (Figure 5.7c).

Table 5.5. Selection of the most precise type of model based on AIC$_c$ values, using the fat-tailed dispersal kernel. Although there is some support for all models, the most likely result is that dispersal and capture probabilities are different between the sexes but that dispersal ability was the same for both years of the study.

Dispersal dependent on	L	k	AIC$_c$	ΔAIC$_c$	w
Neither sex nor year	-1619.0	4	3246.0	1.710	0.181
Sex but not year	-1614.1	8	3244.3	0	0.425
Year but not sex	-1614.5	8	3245.1	0.806	0.284
Both sex and year	-1607.3	16	3247.0	2.712	0.110

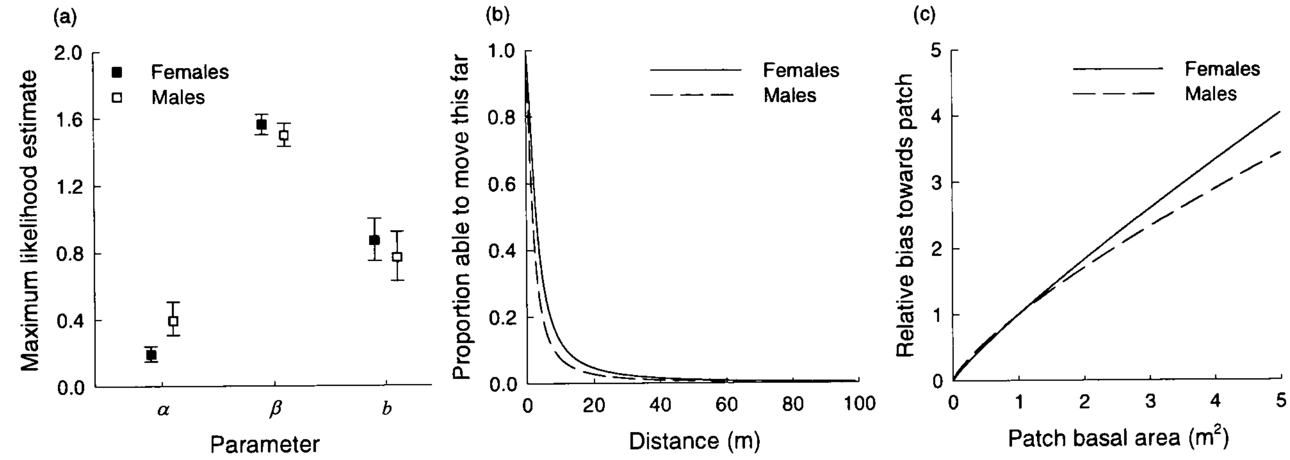


Figure 5.7. (a) Parameter estimates for the fat-tailed kernel model for both years combined but males and females fitted separately, with error bars indicating 2-unit support intervals. (b) The resultant dispersal kernels, which bear close similarity to the histogram of dispersal in Figure 5.5 and (c) relationships between patch size and relative attractiveness for both sexes.

5.3.2.3 Model fit

From the dispersal scenario selected in the above analysis, observed and model-predicted numbers of emigrant males and females at each patch (excluding cases without data) were strongly correlated (Pearson's product moment correlation with significance assessed by a randomisation test, N=87, r=0.821, P<0.001), as were the numbers of immigrants (N=128, r=0.589, P<0.001) (Figure 5.8). Observed and expected numbers of dispersing males and females between each visit were also very strongly correlated (N=47, r=0.960, P<0.001) indicating that the apparent phenology of dispersal was an artefact of changes in the landscape such as mowing, flooding and patch growth (Figure 5.9).

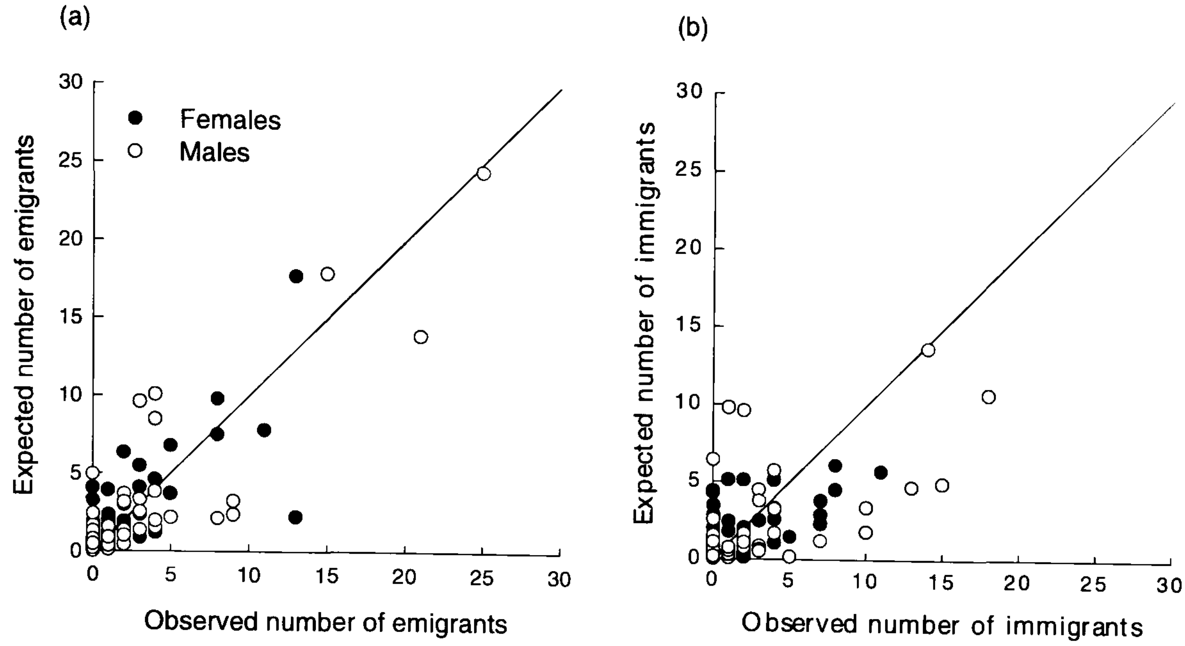


Figure 5.8. Observed and expected numbers of (a) emigrant and (b) immigrant beetles at each patch throughout each year of the study. Black lines show equivalence between observed and expected.

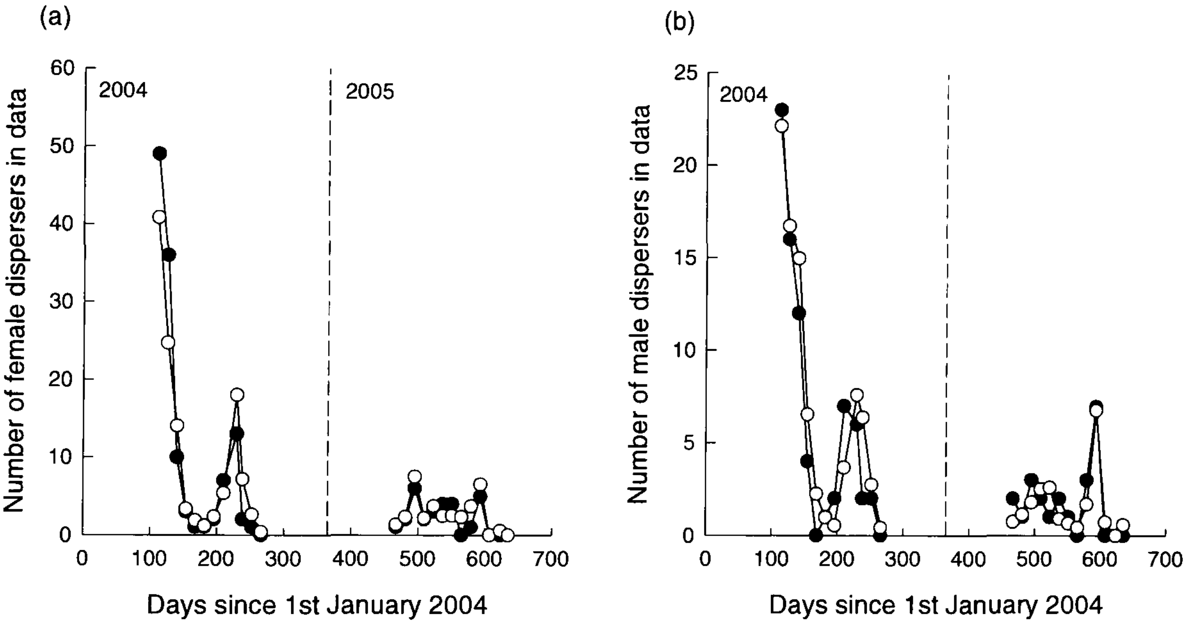


Figure 5.9. Observed (filled circles) and expected (open circles) numbers of dispersing (a) female and (b) male beetles in the dataset over every interval between visits in both years. The close match between observed and expected suggests that the apparent phenology of dispersal in Figure 5.3b is caused by changes in landscape structure rather than behaviour.

5.4 Discussion

The patterns of tansy beetle dispersal uncovered in the mark-recapture study build up a picture of an organism adopting simple behavioural strategies. Dispersal (inter-patch movement) was not biased up or down the river and beetles observed dispersing on multiple occasions often moved back into tansy patches that they previously visited. Rates of inter-patch movement did not differ between years but were higher in females, over longer time periods and when flooding occurred. Emigration from small patches was more common but adult beetle density did not affect the likelihood of dispersing. Net displacements during dispersal were not influenced by beetle sex, the year or the interval between recaptures. Most dispersal distances were less than 100 m but the distribution was highly leptokurtic and right-skewed such that a minority made longer-distance movements, up to a maximum of 856 m.

These results may be those expected for an organism whose dispersal is driven by day-to-day foraging movements causing it to stray from its host-plant, which would explain the lack of inherent dispersal seasonality (Figure 5.9) compared to the extreme seasonality in the beetle's lifecycle. Increased emigration from smaller patches would result from increased perimeter-to-area ratios (Englund and Hambäck 2004) and is well documented for beetle-host-plant systems (Kareiva 1985; Turchin 1986; but see Bach 1988; Matter 1997). A possible cause of the female-biased dispersal rate is their larger body size, which may have affected behaviour inside patches or at their edges, although males and females achieved similar dispersal distances so any such differences may not exist while in the inter-patch matrix. Here, their inability to detect tansy (Sivell 2003; Chapter 4) means they will wander blindly until they starve, suffer predation or reach a patch. Most forays where the beetle survives will result in the individual ending up inside the same patch it left although a minority will lead to successful dispersal.

The dispersal model was based on this concept of dispersal resulting from foraging movement. All host-plants at the site 'competed' for individuals on the basis of their distance from the source patch (scaled by dispersal ability using a dispersal kernel) and their area (as larger patches are larger targets to dispersers) so that landscape structure was accounted for in determining achieved dispersal distances. In the model, emigration was considered implicitly rather than explicitly because it was likely that all beetles would leave their patches at some point over the two weeks between mark-recapture visits. However, source patches competed to retain their beetles and smaller ones had weaker competitive ability leading to higher achieved emigration rates. This type of approach is preferable to simply regressing functions onto empirical distributions of dispersal distances collected in fragmented landscapes (e.g. Hill *et al.* 1996; Baguette 2003) as recorded distances will depend on the landscape that is encountered.

In common with other such models (Hanski *et al.* 2000), I used centre-to-centre inter-patch distances and gave intra-patch movements distances of 0 m. I feel this to be reasonable because I do not consider movement within a host plant patch to be dispersal and intra-patch movement could only be small relative to the inter-patch distances in the patch network (just 9 of 796 inter-patch distances were less than the maximum intra-patch distance in that year). Intra-patch movements were not incorporated in the model because the handling of recaptured beetles meant they were disturbed and moved before being placed back into the centre of their patch. This will be a feature of the majority of insect mark-recapture studies so treating patches as points may often be necessary.

When the model was fitted to the data with different forms for the dispersal kernel (Table 5.1), the Gaussian kernel, which is expected from diffusion theory (Turchin 1998), and the negative exponential, which is commonly used in spatial ecological models (e.g. Biederman 2004; Moilanen 2004), performed poorly because of their inability to account for the long-distance dispersal in the data. Of the remaining candidates, the fat-tailed kernel gave the best fit in all cases. The inverse power function, commonly fitted to empirical datasets (Hill *et al.* 1996; Baguette 2003) and an extended version of the negative exponential performed similarly overall, although the inverse power function gave a somewhat better fit for males in 2004. Using the fat-tailed kernel, the model tended to suggest that females were slightly more dispersive than males but that dispersal ability did not differ between years and gave good matches to the observed patterns of emigration, immigration and the phenology of dispersal. However, the fitted kernels for the two sexes were quite similar, indeed their 2-unit support intervals overlapped, which could explain why the observed dispersal distances of males and females were not significantly different and why the conclusion of sex-difference in the dispersal kernel was not more robust.

Two causes of fat-tailed dispersal kernels have previously been identified – heterogeneous movement behaviour where the more mobile individuals are responsible for the long-distance dispersal (Skalski and Gilliam 2003) and semi-permeable habitat patch boundaries where individuals that cross the boundary quickly may travel far (Morales 2002). The heterogeneous behaviour model predicts that individuals will have Gaussian kernels, but that these will vary among the population and sum to a fat-tailed kernel overall. In the habitat boundary model, where all individuals behave according to the same rules, all individuals inside any one patch will have the same fat-tailed kernel, while individuals in different sized and shaped patches will have different kernels based on how often the boundary is encountered and its permeability. If however some individuals are more adept at crossing patch boundaries than others, then all individuals will have unique patch-dependent fat-tailed kernels.

Unfortunately, my data do not permit an analysis of any heterogeneity in tansy beetle dispersal behaviour because of a limited numbers of captures per individual (of the 2226 marked beetles only 338 had three or more captures, giving the possibility of more than one observed movement). Correlation between successive movements of the same individual may not indicate heterogeneity anyway, because dispersal is highly localised and strongly dependent on landscape structure. Therefore, after one movement, an individual will generally be in a similar landscape to the one it left, so may make a similar second movement for this reason. One possible way of addressing heterogeneity would be to adapt the current model by treating the parameters of each individual as random variables drawn from, for example, a normal distribution and fitting the distribution parameters to the data. The estimated variance of the distribution will represent the level of heterogeneity. However, such an analysis would require multiple observations of the same individual moving in many locations, as are perhaps more often obtained in radio-tracking studies of large animals than mark-recapture studies of insects.

The dispersal model did not include mortality during dispersal, so in fact I fitted 'successful dispersal' kernels. Mortality is likely to increase with the distance travelled (Clobert *et al.* 2004) so I would expect the tail of long-distance dispersal of a true kernel to be higher than that observed. Thus, the finding that fat-tailed kernels outperformed less fat-tailed ones is robust. The Virtual Migration Model (Hanski *et al.* 2000) includes mortality as a function of patch connectivity, since dispersal from isolated patches will generally be further and thus riskier. This tactic could potentially be used to estimate dispersal mortality from these results. A multi-state mark-recapture model where study patches are states (Hestbeck *et al.* 1991) could be set up with inter-patch transition probabilities between each visit fixed to those predicted by the current model. Assuming dispersal mortality is related to patch connectivity, which can be calculated from the results of the model (see Moilanen and Nieminen 2002), patch-specific survival rates could be estimated from the mark-recapture data as a function of patch connectivity using a constrained linear model (e.g. Lebreton *et al.* 2003).

The structural assumptions of the dispersal model could also be altered to suit the biology of other species. Emigration could be included overtly as a simple function of patch area (Equation 5.3). Further extensions could include replacing the patch area terms with alternative measures of size or quality, perimeter-to-area ratios or the density of conspecifics and if the species was polyphagous, modifiers for plant species could be included. Dispersal probabilities could be made conditional on previous locations, such that individuals may avoid patches they have recently visited. However, this would require multiple recaptures and marking from eclosion and in this case, it seems unlikely that *C. graminis*, which cannot detect tansy at distance (Chapter 4), would be capable of employing such a strategy. The dispersal kernel could also be altered to include multiple modes of movement such as flying and walking, which are likely to operate over different spatial scales. Data permitting, model performance with and without these modifications would be compared to indicate whether the extra complications were necessary. Given the range of adaptations that could be implemented relatively easily, I suggest that this type of model is a flexible tool that could be applied generally to data on other insect herbivores.

Although mechanistic models are desirable, pattern-oriented phenomenological approaches, such as the one taken here, are able to reveal important influences on dispersal, in this case sex, patch area and inter-patch distance. Also, they are more efficient to implement in spatial models and so therefore will play an important role in the study of dispersal. Many models use Gaussian or negative exponential kernels, presumably because of their simplicity and relationship to theories of animal movement. This study adds to the body of evidence that 'fatter-tailed' kernels are more realistic (Hill *et al.* 1996; Kot *et al.* 1996; Thomas and Hanski 1997; Baguette 2003; Skarpaas *et al.* 2004) and suggests that modellers take account of this.

Chapter 6: Population genetic structure

6.1 Introduction

A consequence of limited dispersal is population genetic structure, which can be quantified by exploring spatial patterns of neutral genetic diversity (Beebee and Rowe 2004; Lowe *et al.* 2004; see Chapter 1). Since such patterns are related to gene flow distances and hence dispersal, investigating the genetic structure of *Chrysolina graminis* forms a natural extension to the preceding chapters. In order to do this, reliable neutral genetic markers must be developed at multiple loci. Two types of marker are considered in this chapter – allozymes and microsatellites, the nature and utility of which are described below.

The advent of allozyme electrophoresis as a method of quantifying genetic diversity in the late 1950s led to the development of the field of molecular ecology (Beebee and Rowe 2004). Allozymes are variants of the same enzyme, encoded in the genome at the same locus, which differ in electrophoretic mobility because of variation in their amino acid sequences (Richardson *et al.* 1986). Thus, individual phenotypes can be scored by measuring how far their allozymes migrate during electrophoresis, and this is interpreted as a genotype. Allozymes have several advantages over other markers. For example, knowledge of genetic sequences is not required and established methodologies for scoring more then 100 loci (Richardson *et al.* 1986; Hebert and Beaton 1993) mean that development costs are typically low and many loci can be considered. However, levels of variation at allozyme loci may be lower for other types of marker and the banding patterns on electrophoretic gels can be complex and difficult to interpret (Lowe *et al.* 2004). Also, since allozymes are functioning enzymes, the variation at these loci may not be selectively neutral (Mitton 1997), while the majority of statistical analyses for genetic data assume neutrality.

Microsatellites, also known as simple sequence repeats (SSRs), simple tandem repeats (STRs) and variable number tandem repeats (VNTRs), are alternative markers that are being used increasingly frequently in population genetic studies (Scribner and Pearce 2000). They typically consist of 10-50 repeats of a 2-5 base pair motif, which are non-coding (and hence neutral, unless closely linked to coding sequences) and exhibit high levels of length polymorphism (Lowe *et al.* 2004). Length polymorphism probably arises in a largely stepwise fashion due to slippage during DNA replication (Schlötterer and Tautz 1992), the rate of which may depend on the repeat motif and any interruptions found within the microsatellite sequence (Scribner and Pearce 2000). This mutation mechanism is different to the way in which allozyme polymorphism is thought to develop and can cause homoplasy (the same allele arising through multiple mutations) (Schlötterer *et al.* 1998). Thus, a stepwise mutation mechanism necessitates different statistical treatment of microsatellite data; for example, the use of R_{ST} to describe population structure (Slatkin 1995; see Chapter 1).

Although their variability makes microsatellites powerful genetic markers, a major drawback to their use is the need to isolate specific markers for each species under study. To genotype a microsatellite locus it must be amplified from the genome with a polymerase chain reaction (PCR). This requires primers – a pair of oligonucleotides that are complementary to sequences flanking the microsatellite region such that they will bind on either side of it, allowing replication of the microsatellite region but not the rest of the genome. Successful primer pairs must have similar annealing temperatures and sufficient specificity that unwanted amplification is minimal. Since the flanking regions from which primers are developed may also be non-coding and so poorly conserved through evolutionary time, novel primers are required for most species, although there are examples of cross-amplification in species separated by as much as 470 million years (Rico *et al.* 1996). Primer development is expensive and time-consuming, so can present a major challenge when undertaking such studies (Zane *et al.* 2002).

There are a number of reasons to suspect that significant population structure will be evident for *C. graminis*. Firstly, movements and dispersal are spatially limited (Chapters 4 and 5) so gene flow between areas separated by more than a few hundred metres will be low, potentially allowing populations to diverge at neutral loci through drift and at non-neutral loci by both drift and local selection.

Theoretically, if populations exchange fewer than one individual per generation then differentiation at neutral loci will occur (Allendorf 1983). Given the results of Chapter 5, populations on the same side of the river within ~1 km of one another may therefore be genetically similar, while divergence will occur for populations further apart. Since *C. graminis* is flightless, the River Ouse should form a barrier to dispersal, which may cause even geographically close populations to diverge if on opposite riverbanks. Furthermore, its small and highly fluctuating local populations will accelerate drift, the rate of which being inversely proportional to population size (Lowe *et al.* 2004). Founder effects associated with repeated local extinctions and colonisations will have similar effects (Pannell and Charlesworth 2000). Thus, alleles in unconnected small beetle populations will drift independently and rapidly to different frequencies, such that allele frequencies will differ and population genetic structure will have developed. Populations that are further apart will be more differentiated as gene flow between them will be lower – leading to the development of 'isolation-by-distance' (IBD) (Wright 1943).

In this chapter, I aim to relate *C. graminis* genetic divergence to dispersal ability. In order to do this, the development of both allozyme and microsatellite loci was attempted. Genotype data from successfully developed markers was collected from beetles sampled at eight localities along the Ouse. From these data, overall population structure is quantified at each locus, and tests of population structure and linkage between the loci are performed. Population genetic structure is analysed under an IBD framework, using Mantel tests to assess the relationship between geographic distance and pairwise F_{ST} and R_{ST} values. These statistics quantify the level of divergence among populations by describing the reduction in heterozygosity within samples relative to the overall population, attributable to drift in neutral loci under infinite allele or stepwise mutation models respectively (Lowe *et al.* 2004). Finally population-pairs are split into groups dependent on the expected level dispersal among them (from the results of Chapter 5) and the statistical significances of differences in F_{ST} and R_{ST} from each group are tested.

6.2 Allozyme electrophoresis

6.2.1 Materials and methods

Allozyme analysis was conducted at 21 loci using cellulose acetate electrophoresis following the methods outlined in Hebert and Beaton (1993) and Richardson *et al.* (1986). Allozyme extracts were prepared from wild-caught or captive-sourced adult beetles killed by freezing at -80 °C. Beetles were starved for two days prior to killing to avoid any effects that undigested tansy tissue in the sample might have on enzymatic activity (Oxford 1975). After storage at this temperature, beetle heads and thoraxes were dissected and homogenised in 70 µl of extraction buffer (100 ml Tris HCl pH 8.0 buffer and 0.02 g NADP). The suspended material was centrifuged at 6000 rpm and 4 °C for 5 minutes and the supernatant, containing active beetle enzymes, was retained and divided into several aliquots and stored at -20 °C.

Electrophoresis of thawed extracts was performed with a Helena Super Z-12 Applicator Kit (Helena Laboratories) and a standard electrophoresis tank. For this, cellulose acetate plates were soaked in one of two running buffers; Tris Glycine pH 8.5 (3.0 g Trizma base and 14.4 g Glycine in 1 l of distilled water, adjusted to pH with 1 M HCl) or Tris Maleate pH 7.8 (6.06 g Trizma base, 0.37 g Na_2EDTA, 0.095 g $MgCl_2$ and 2.32 g maleic acid in 1 l of distilled water, adjusted to pH with 1 M HCl), dependent on the locus. For some loci, samples were mixed with 0.5% β-mercaptoethanol, which can stabilise the active enzyme and reduce secondary banding (Richardson *et al.* 1986).

A row of beetle extracts was applied to the centre of a cellulose acetate plate before electrophoresis at 200 V and 4 °C. After the plate had run for 30-40 minutes (dependent on the enzyme), it was removed from the electrophoresis tank and an enzyme-specific stain solution, suspended in 2 ml of melted agar (2 g of agar powder in 120 ml of distilled water) was poured evenly over the plate. Stain recipes were taken from Hebert and Beaton (1993) and Richardson *et al.* (1986). The plate was incubated in darkness until banding appeared where the enzyme had reacted with the stain or no activity was detected after several hours. The agar-stain layer was then removed from the plate allowing any bands to be scored according to how far each isozyme had migrated.

One locus (α-Amylase) required a different staining procedure (Hebert and Beaton 1993). Here, a petri dish was filled with melted agar/starch solution and allowed to cool to room temperature and set. The cellulose acetate plate was removed from the electrophoresis tank, pressed into the starch/agar dish and incubated at room temperature for 10 minutes. The plate was then peeled from the agar and immersed in iodine solution, which stains the parts containing starch black, leaving the amylase bands unstained.

6.2.2 Results

Electrophoresis was generally unsuccessful, despite multiple attempts and experimentation with electrophoretic conditions (Table 6.1). Of the 21 loci trialled, eight (AMY, GPI, IDH, MDH, ME, MPI, 6PGDH and PGM) yielded interpretable banding patterns, and some of these tended to be inconsistent. Polymorphism was detected in four loci, with a maximum of three alleles at any one locus. Given the inconsistency and low levels of variation, it was decided that allozyme data would be insufficient for the purposes of this study and so allozyme electrophoresis was not pursued further.

Table 6.1. Summary of the results of trials of allozyme loci on *C. graminis* adults. The running buffers mentioned are Tris Glycine pH 8.5 (TG) and Tris Maleate pH 7.8 (TM).

Enzyme	**Results**
Adenylate kinase (AK)	Poor staining
Alcohol dehydrogenase (ADH) with ethanol	Inconsistent staining and uneven bands
Alcohol dehydrogenase (ADH) with octanol	Inconsistent staining and uninterpretable bands
Aldehyde dehydrogenase (AD)	Poor and uninterpretable banding
Aldehyde oxidase (AO)	Poor and uneven banding and seemed monomorphic
α-Amylase (AMY)	Consistent and interpretable bands when plate is run in TG for 25 minutes. Monomeric locus with 2 alleles detected.
Aspartate amiro transferase (AAT)	Weak staining
Fumarate hydratase (FUM)	Poor staining
Glucose-6-phosphate dehydrogenase (G6PDH)	Inconsistent staining and uninterpretable bands
Glucose-6-phosphate isomerase (GPI)	Inconsistent staining with interpretable bands when plate is run in TG for 40 minutes. No evidence of polymorphism
Hexokinase (HEX)	Poor staining
Isocitrate dehydrogenase (IDH)	Strong stain and good bands when plate run in TM for 30 minutes. No polymorphism detected.
Lactate dehydrogenase (LDH)	Inconsistent staining and poor banding
Malate dehydrogenase (MDH)	Strong stain and good bands when plates run in TM for 30 minutes, although this easily overstains and often gave uninterpretable and uneven banding. No polymorphism detected.
Malate dehydrogenase NADP+ (ME)	Strong staining, though often with uninterpretable banding when plate is run in TM for 40 minutes. No evidence of polymorphism where plate is scoreable.
Mannose-6-phosphate isomerase (MPI)	Inconsistent staining but may get interpretable bands when plate is run for 40 minutes in TM. Monomeric locus with two alleles detected
Phosphoglucomutase (PGM)	Consistent and interpretable bands when plate is run in TG for 40 minutes. Monomeric locus with three alleles found.
6-Phosphogluconate dehydrogenase (6PGDH)	Inconsistent but interpretable bands when plate is run in TG buffer for 25 minutes. Dimeric locus with two alleles found.
Trehalase (TRE)	Overstains with uninterpretable banding
Unknown dehydrogenase	Stains with β-mercaptoethanol but gives uninterpretable bands
Xanthine dehydrogenase (XDH)	Inconsistent and slow staining with uninterpretable bands

6.3 Microsatellites

6.3.1 Materials and methods

6.3.1.1 Microsatellite isolation and characterisation

An enriched library was constructed by ecogenics GmbH (Zurich, Switzerland) from size-selected genomic DNA ligated into SAULA/SAULB-linker (Armour *et al.* 1994) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschi *et al.* 2000a, b). Of 372 recombinant colonies screened, 206 gave a positive signal after hybridization. Plasmids from 50 positive clones were sequenced. From these sequences, primers were designed and tested for 13 microsatellites, first using unlabelled primers. If this was successful (gave clear bands after electrophoresis on a 1.5% agarose gel), PCR was carried out using primers that were fluorescently labelled at the 5' end (Applied Biosystems). PCR followed a HotStart procedure in a 10 μl reaction containing 2 μl of genomic DNA (ngμl^{-1}), 4.7 μl of filtered distilled water, 1 μl 10X PCR buffer (Qiagen) containing 1.5 mM $MgCl_2$, 1 μl 1.5 mM PCR nucleotide mix (Promega), 0.6 μl of each primer (5 μM) and 0.1 μl HotStarTaq DNA polymerase (Qiagen). The PCR program began with 95 °C for 15 minutes, followed by 30 cycles of 40 s at the locus-specific annealing temperature, 40 s at 72 °C and 40 s at 95 °C. The program was completed with a further 1 min at the annealing temperature, 8 min at 72 °C and 30 min at 4 °C. Annealing temperatures were varied between 53 and 60 °C to try to optimise the PCR for each primer. Labelled PCR products were evaluated with a 3730xl DNA Analyser (Applied Biosystems) at the Department of Biochemistry, University of Oxford using a LIZ-500 size standard and allele sizes determined with GeneMapper 3.7 (Applied Biosystems).

6.3.1.2 Collection and preparation of samples

Adult and larval *C. graminis* were collected from wild populations at eight sites in June 2005 (Figure 6.1). Collection sites were defined as 100 m lengths of the bank, separated by a minimum of 50 m of intervening bank or the River Ouse. Tansy plants growing within each site were searched for individuals, with the aim of collecting 30 beetles per site. This was accomplished for all but the Clifton Ings site (Figure 6.1), where only 29 individuals were found. The centre-to-centre inter-site distances between samples from the same side of the river ranged from 180 m to 7972 m. From the results of Chapter 5, population differentiation within a site (100 m stretch of bank) should not occur as movement between patches will be common. The closest sites should also be linked by some dispersal and thus gene flow, while the most distant are likely to be completely isolated and will diverge.

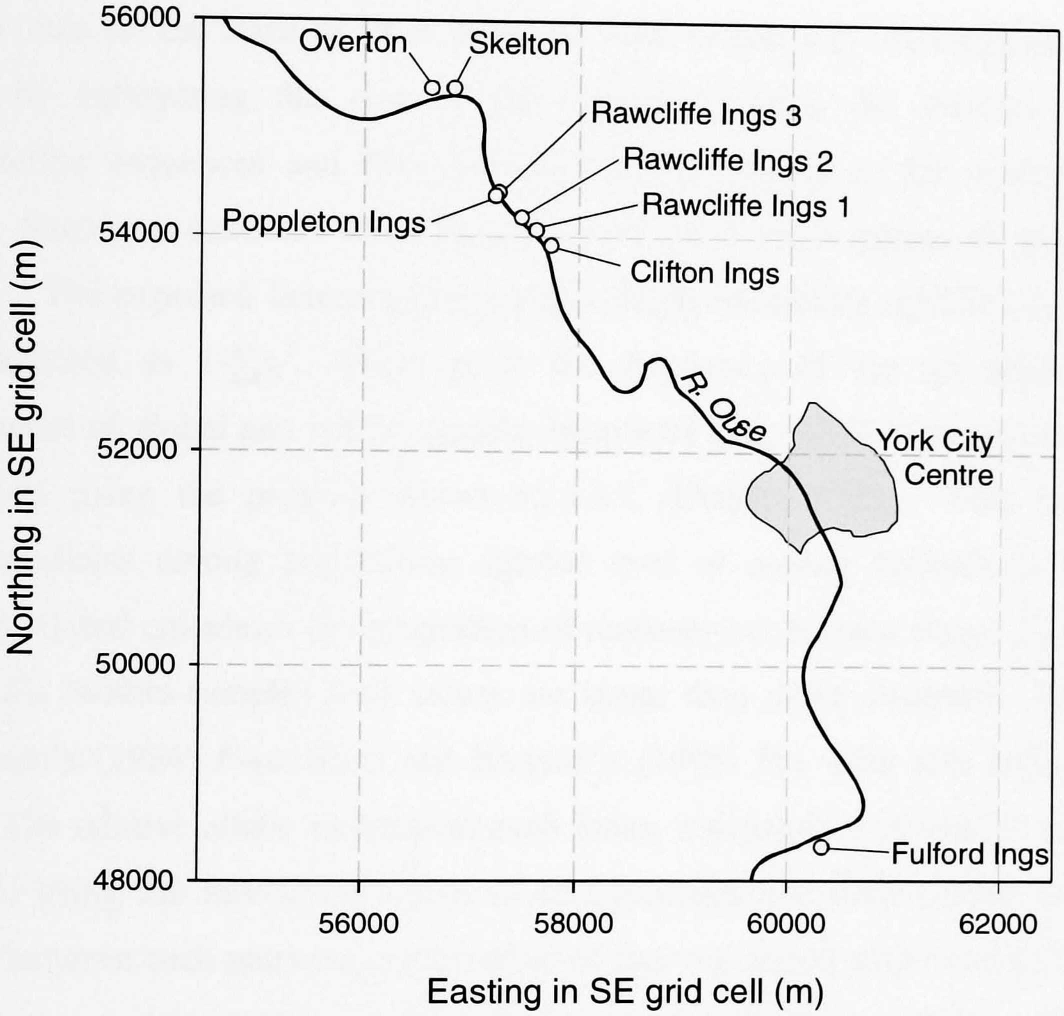


Figure 6.1. Map showing the sites where individuals were collected for microsatellite analysis.

After collection, adults and larvae were brought back to the laboratory, where they were kept in separate tubes in a constant temperature cabinet at 18 °C with 16:8 hours of light to dark each day. Adults were starved for two days prior to killing by immersion in cold ethanol (-20 °C). Larvae were provided with fresh tansy leaflets on an *ad hoc* basis and reared until they achieved a large size and appeared close to pupation. At this point they were starved for two days and killed in the same way as the adults. DNA was extracted from dissected heads and thoraxes using Qiagen DNEasy kits, following the animal tissues protocol. DNA extraction concentrations were ascertained with an ND-1000 spectrophotometer (NanoDrop Technologies) and the extracts diluted to 5 ngul^{-1} for PCR. Individuals were subjected to DNA extraction and PCR in a random order and without knowledge of which population they came from, to eliminate any possible bias.

6.3.1.3 Statistical analysis

The raw data on the sizes of PCR products were scored into microsatellite repeat lengths by subtracting the size of the regions between the primers and the microsatellite sequences and then rounding the remainder to the nearest repeat length. Summary statistics were calculated for each locus across all population samples. The expected heterozygosity under Hardy-Weinberg equilibrium (HWE) was calculated as $1-\sum p_i^2$, where p_i is the frequency of the ith allele. The significances of global and within-sample departures from HWE were assessed with exact tests using the program FSTAT v2.9.3.2 (Goudet 1995). This randomly permutes alleles among populations (global test) or among individuals (within-sample test) and calculates the proportion of randomised datasets whose F_{IT} (global test) or F_{IS} (within-samples test) values are larger than those observed. Weir and Cockerham's (1984) F-statistics and Rousset's (1996) R_{ST} were also estimated in FSTAT. The relative allelic richness at each locus, independent of sample size, was estimated using the rarefaction index of El Mousadik and Petit (1996) in FSTAT. Linkage between each pairwise combination of loci was tested with exact tests (1000 randomisations) in GENEPOP v.3.4 (Raymond and Rousset 1995). Tests of population differentiation (not assuming HWE) were also performed in FSTAT with an exact G-test (Goudet *et al.* 1996). Multilocus F_{IS} values were calculated for each population sample with FSTAT. Since F_{IS} (a measure of the level of inbreeding) may be related to the number of tansy patches within a site, correlation coefficients between these two statistics and the number of patches were calculated.

Isolation-by-distance (IBD) analyses were carried out on both pairwise F_{ST} and R_{ST} values. Weir and Cockerham's (1984) multilocus F_{ST} estimates were calculated with FSTAT and Slatkin's (1995) multilocus R_{ST} was calculated with the program

MICROSAT (http://hpgl.stanford.edu/projects/microsat/). These were regressed against log-transformed geographical distances between the populations in the IBD software package (Bohonak 2002), which uses Reduced Major Axis regression and assesses significance with Mantel tests. Log-transformed distances were used since these are the most appropriate for IBD in one-dimensional ranges (Rousset 1997), which riverbanks approximate at large scales. Isolation-by-distance analyses were conducted both with and without the one population on the opposite side of the river to the other seven (Poppleton Ings, Figure 6.1), since the geographical distance across the river may not be indicative of the barrier it presents to gene flow compared to the same distance along the riverbank.

The correspondence between the recorded population differentiation and the demographically established dispersal ability of tansy beetles (Chapter 5) was then tested. To do this, population pairs were categorised into four groups, according to the likely level of dispersal between them. The groups were as follows,

- Regularly expected to exchange migrants (<1000 m apart on the same bank).
- Just outside the dispersal range (1000-2000 m apart on the same bank).
- Well out of range (>2000 m apart on the same bank).
- Separated by the river so unlikely to exchange migrants.

The mean pairwise F_{ST} and R_{ST} values were calculated for each group and the significance of this difference was assessed with a randomisation test, whereby the mean values were permuted among members of each pair of groups 10000 times in a self-coded C++ program.

6.3.2 Results

6.3.2.1 DNA extraction

DNA extractions always yielded higher concentrations of genomic DNA than were required for PCR. However, the amount of DNA extracted from adult beetles was significantly lower than that from larvae (Mann-Whitney U test, N=239, U=-8.068, P<0.001). The mean (± standard deviation) extraction concentrations were 97.2 (± 35.5) ngul^{-1} for larvae and 65.0 (± 26.0) ngul^{-1} for adults.

6.3.2.2 Primer development

Of the 14 loci for which primers were tested, six yielded scoreable PCR products (Table 6.2). The results of PCR with these loci are shown in Table 6.3. Locus P1 was rejected from further analyses because of poor success rate in amplification. Locus P7 was also discarded because it was almost monomorphic (the second allele was located only once). I did not include locus P11 in the full study because although it had a relatively high PCR success rate and showed polymorphism, replicated PCRs of a number of individuals were consistently dominated by a short-length product, not characteristic of a microsatellite, rather than the desired amplification. Thus, null alleles may be present and using this locus may be problematic.

Table 6.2. Microsatellite loci for which primers were tested, with a summary of the outcome of attempted PCR. The repeat motif is that detected in the sequenced clone and T_m is each primer' melting temperature.

Loci	Repeat motif	Primer sequence (5'-3')	T_m °C	Insert size (bp)	Results
CH01	[GA]$_{26}$	L: GGTAACCACTTGGGAATAGT R: TTCTACGAGCTGTTTTCTGT	L: 53.16 R: 52.81	193	Consistently scoreable and polymorphic
CH02	[CA]$_{18}$	L: CAAGTTGAAATTCCAAGAAA R: AGCTAATGACTCAGATGCAC	L: 53.04 R: 52.91	150	No amplification
P1	[GT]$_{36}$	L: ACTAAAAATCGGGGTGTATC R: TAGGAAGACCCCTTAATGAT	L: 53.31 R: 52.93	248	Inconsistent amplification, but polymorphic
P2	[GT]$_{35}$	L: GGATCCATAACATACAGTGG R: GGATCCATAACATACAGTGG	L: 52.69 R: 53.04	201	No amplification with labelled primers
P3	[GA]$_{33}$	L: AAACAGCTATGACCATGATT R: GGGATCCCGTTAATCTAAG	L: 52.17 R: 53.74	195	Excessive non-specific amplification
P4	[CA]$_{32}$	L: GGTCGACTCTAGAGGATCAG R: TATTCCTAAGACCAATTGAA	L: 53.91 R: 50.02	238	Excessive non-specific amplification
P5	[GT]$_{30}$	L: AAATTTGATAGCCATGTGAA R: ACAATTTTCCGTATTTGTGA	L: 53.27 R: 53.19	183	Consistently scoreable and polymorphic
P6	[CT]$_{28}$	L: TTAGAACAGCTGGTTTATGG R: GTTTCTTTGGACATGAACTG	L: 53.12 R: 53.09	193	Consistently scoreable and polymorphic
P7	[GA]$_{26}$	L: CCTCGTCTATTGCTTTCATA R: GGATCTCGTGTCTATTTCCT	L: 53.19 R: 53.29	176	Consistently scoreable but monomorphic
P8	[CT]$_{26}$	L: CGTATCAAAACAAGTCCAAG R: GAAAAATGGCAGGTACATAA	L: 53.41 R: 52.91	242	Excessive non-specific amplification
P9	[CT]$_{21}$	L: GATTCCCCAATAAGTCAGAT R: TGTTTCTCTGAAGTTGGAAG	L: 53.09 R: 52.97	182	Little amplification
P10	[CA]$_{20}$	L: GGAGAAGCTTGAAGAAAACT R: GTTTTTCATTTGTTGTCGTT	L: 53.03 R: 52.80	176	Little amplification
P11	[CT]$_{17}$	L: GGATCAAAAGCATAAAAGTG R: AAAGCCACAAAATAAAATCA	L: 52.61 R: 52.70	150	Inconsistent amplification but polymorphic
P12	[TC]$_{19}$	L: GATGTCCAGGAAGACTTGTA R: TTCCACCTATATTCCAACAA	L: 52.51 R: 53.11	227	Excessive non-specific amplification

Table 6.3. Results of PCR with the primers that successfully amplified microsatellite loci. T_A is the optimised annealing temperature and N is the number of beetles tested. H_O and H_E are the observed and expected (at Hardy-Weinberg equilibrium) heterozygosities across all populations. Those loci used to analyse beetle population structure are also indicated.

Locus	T_A (°C)	N	PCR success	Number of alleles	Size range (repeats)	H_O	H_E	Used further?
CH01	53	239	97%	7	26-32	0.273	0.667	Yes
P1	55	239	32%	6	35-40	0.368	0.613	No
P5	53	239	96%	7	27-33	0.261	0.712	Yes
P6	55	239	74%	6	26-30	0.181	0.618	Yes
P7	53	59	100%	2	27-28	0.017	0.017	No
P11	53	35	69%	3	17-19	0.391	0.369	No

The alleles at the three useful loci had similar size ranges and within the range of allele lengths, all possible sizes were recorded (Figure 6.2). Evidence of heterozygote deficiency was found for each useful locus (Table 6.3), which led to significant overall deviations from Hardy-Weinberg equilibria (Table 6.4). Summary statistics for each locus across all populations (Table 6.4) indicated that although F_{ST} values for all loci were quite low, significant population structure was evident. Global R_{ST} values were higher and more consistent across loci than F_{ST}, which may be because this statistic is developed for a stepwise mutation model and so assigns greater divergence between large and small alleles than F_{ST}. F_{IS} and F_{IT} values were quite high, indicating either within-sample population structuring (Wahlund effects) or high levels of inbreeding within subpopulations and over all samples. Allelic richness, as estimated by rarefaction was similar in all three loci and there was no significant linkage between loci (Table 6.5), suggesting they are inherited independently.

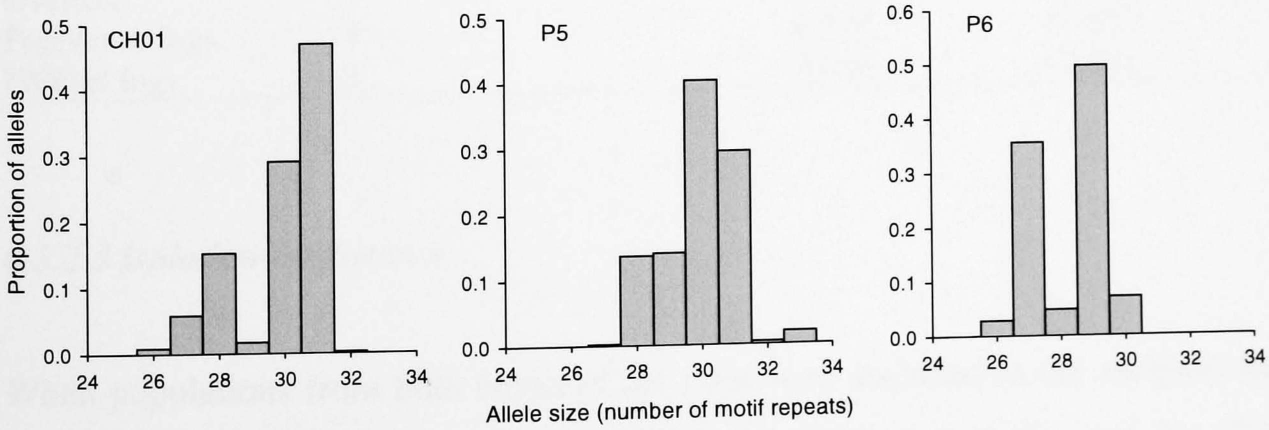


Figure 6.2. The distributions of allele sizes at each of the useful loci (Table 6.3).

Table 6.4. Summary statistics for the three useful loci from Table 6.3 calculated across all sampled populations. *F*-statistics are calculated using Weir and Cockerham's (1984) estimators (weighted by sample size in each population) and R_{ST} is calculated with Rousset's (1996) estimator (each locus weighted by its allelic variance). *P*(HWE) are the *P*-values from exact tests of Hardy-Weinberg equilibrium across populations. *P*(PS) are the *P*-values for exact tests where the null hypothesis is no population structure.

Locus	F_{ST}	F_{IS}	F_{IT}	R_{ST}	Allelic richness	*P*(HWE)	*P*(PS)
CH01	0.086	0.561	0.599	0.267	4.422	<0.001	<0.001
P5	0.111	0.597	0.642	0.290	4.538	<0.001	<0.001
P6	0.205	0.643	0.716	0.187	4.135	<0.001	<0.001
All loci	0.132	0.598	0.651	0.251	-	<0.001	<0.001

Table 6.5. Results of exact tests of linkage disequilibria among the three useful microsatellite loci.

Locus 1	Locus 2	χ^2_{16}	*P*
CH01	P5	14.16	0.587
CH01	P6	20.99	0.179
P5	P6	12.21	0.729

Allele frequencies within all samples deviated significantly from HWE (Table 6.6). However, there was no relationship between the number of tansy patches at the site and F_{IS} (Pearson's correlation, N=8, r=-0.254, P=0.543) although the sample size was too small to reveal anything other than a very strong correlation.

Table 6.6. Combined summary statistics for the three useful loci within each sample. The statistics reported are as in Table 6.4.

Sample	Number of patches	F_{IS}	*P*(HWE)
Clifton Ings	1	0.565	<0.001
Rawcliffe Ings 1	6	0.508	<0.001
Rawcliffe Ings 2	4	0.549	<0.001
Rawcliffe Ings 3	3	0.596	<0.001
Skelton	4	0.696	<0.001
Overton	5	0.450	<0.001
Poppleton Ings	4	0.772	<0.001
Fulford Ings	3	0.646	<0.001

6.3.2.3 Isolation-by-distance

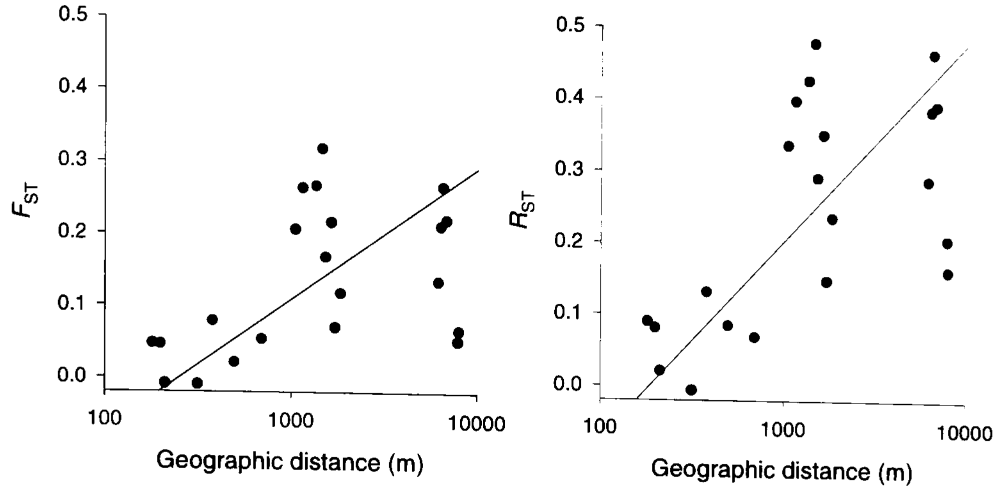
When populations from both banks of the river were included in the analysis, there was evidence of significant isolation-by-distance (IBD) in both F_{ST} and R_{ST} (Table 6.7). When the seven populations from the east bank were considered alone (i.e. the population from the opposite side of the river was excluded), the strength of IBD was increased, both in terms of its significance and its slope (Table 6.7, Figure 6.3).

Table 6.7. Results of isolation-by-distance (IBD) analyses for the seven populations on the same side of the river (east) and the complete dataset including the one population from the west bank. Reduced Major Axis regression was used to predict population differentiation (F_{ST} and R_{ST}) from ($\log_{10}$) geographic distance. Because of spatial non-independence, the significance of the IBD is assessed with Mantel tests. Intercepts and slopes of the regressions, as well as their R^2 are reported. Because only one population on the west bank was sampled, there were insufficient data to perform cross-river IBD analyses.

Populations	Response	N	Mantel statistic	*P*	Intercept	Slope	R^2
East and west	F_{ST}	28	10.986	0.038	-0.363	0.159	0.091
East	F_{ST}	21	9.353	0.011	-0.440	0.183	0.226
East and west	R_{ST}	28	20.112	0.005	-0.533	0.248	0.148
East	R_{ST}	21	16.938	0.005	-0.633	0.279	0.388

From Figure 6.3, it appears that the true relationship between F_{ST} and R_{ST} and ($\log_{10}$) distance may be sigmoidal with population differentiation among populations on the same riverbank saturating to a maximum limit just above 1 km, although the graph contains much scatter. Thus the linear relationship fitted in the IBD analyses of Table 6.7 may not have been realistic. This was explored with Monte Carlo tests, randomising F_{ST} and R_{ST} values among four groups defined based on the dispersal range of the beetle (Table 6.8). Similar relationships were found for both F_{ST} and R_{ST}. For samples from the same side of the river, the differentiation between those within dispersal range was significantly lower than the differentiation between those either just outside or well outside range. However, the differentiation between populations just out of range was not different to that among populations well outside the dispersal range. The differentiation among populations on opposite sides of the river was significantly different to that among those on the same side of the river if they were in dispersal range or just outside it, but populations well outside the range of dispersal were as distinct as those across the river.

(a) Same bank



(b) Opposite banks

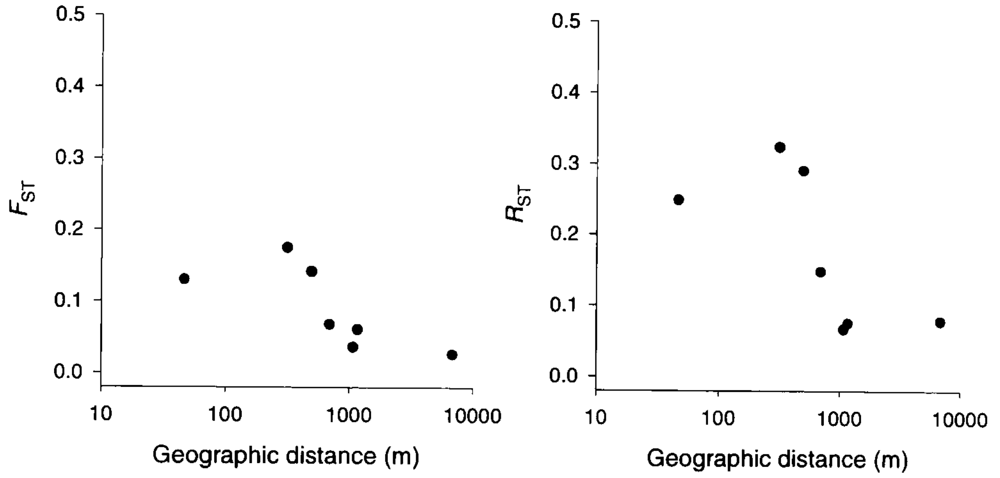


Figure 6.3. Pairwise F_{ST} and R_{ST} between populations on (a) the same and (b) opposite sides of the river plotted against distance. The fitted isolation-by-distance relationships for populations on the same bank are shown.

Table 6.8. P-values for the absolute differences between mean F_{ST} (above and to the right of the diagonal) and R_{ST} (below and to the left of the diagonal) among populations grouped according to the likely levels of dispersal among them. P-values were assessed by 10000 randomisations and those significant at the $P<0.05$ level are highlighted in bold.

Group A	**Group B**			
	Within dispersal range	Just outside dispersal range	Well outside dispersal range	Across the river
Within dispersal range		**<0.001**	**0.004**	**0.041**
Just outside dispersal range	**<0.001**		0.330	**0.012**
Well outside dispersal range	**<0.001**	0.785		0.127
Across the river	**0.039**	**0.016**	0.050	

6.4 Discussion

6.4.1 Marker development

Much effort was devoted to developing selectively neutral genetic markers to assess the population genetic structure of *C. graminis* with less success than was hoped for. Four polymorphic allozyme loci and three microsatellite loci were developed, although because of their greater variability only microsatellites were used to characterise population structure. With hindsight, both types of marker could have been used, but genetic samples were stored in alcohol, which denatures allozymes, since it was wrongly anticipated that a greater number of microsatellite loci would be forthcoming. Nonetheless, as a result of this research, future studies will be able to utilise at least seven markers to investigate the population genetics of *C. graminis*.

A possible reason for the low success of allozyme electrophoresis is that *C. graminis* tissue may contain noxious defensive compounds that interfere with enzymatic activity and thus prevent allozyme staining. Several defensive chemicals have been isolated from other chrysomelids, including *Chrysolina* species (Pasteels *et al.* 1994) and these may be both derived from consumed plant material or directly synthesised by the beetles (Pasteels *et al.* 1977; Daloze and Pasteels 1979; Van Oycke *et al.* 1987; Hilker *et al.* 1992). If *C. graminis* absorbs defensive compounds from tansy it may sequester them in specialist storage tissues to avoid any adverse effects on its own enzymatic activity. Thus, tissue homogenisation during allozyme sample preparation would have brought these into contact with the beetle's enzymes resulting in denaturation or reduced activity. Interestingly, one of the loci that did work was the digestive enzyme amylase, which should be adapted to function in the presence of any tansy secondary chemicals. Richardson *et al.* (1986) recommend including instant coffee granules in the extraction buffer to counteract any defensive compounds, which was not tried in this study and offers a potential avenue for pursuing allozyme research further.

Of 13 microsatellite sequences for which primers were designed, three led to reliably scoreable and polymorphic markers. Undoubtedly other loci could have been developed by testing primers for the remaining 37 sequences, but budgetary and time constraints prevented this from happening. The development of additional loci would mean that more sophisticated multilocus statistical methods could be used, for example population assignment methods to infer migration rates between samples (e.g. Wilson and Rannala 2003). This would have the advantages of assessing cross-river gene flow and producing a same-bank gene flow kernel, which are not really possible with only three loci.

6.4.2 Genetic structure

Using microsatellite data from eight populations, significant departures from Hardy-Weinberg equilibrium were observed in terms of deficiencies of heterozygotes, both globally and within samples. Heterozygote deficit is symptomatic of a variety of processes, including population structuring (Beebee and Rowe 2004; Lowe *et al.* 2004) of which there was evidence at all loci. Inbreeding accompanies population structure (Paxton *et al.* 1996) and the relatively high within-sample F_{IS} values found for *C. graminis* suggested this to occur. Within-sample structuring cannot be discounted as an alternative explanation for the high F_{IS}, although if this were the case it would be expected that it would be related to the number of tansy patches within the collection site, which it was not.

Observed heterozygosity was 30-40% of that expected in all three utilised loci. If loci show variable heterozygote deficit another explanation for this phenomenon may be the presence of null alleles (Voelker *et al.* 1980). These can occur where polymorphism in the primer sequence prevents successful PCR in some cases. Heterozygote individuals at both the primer sequence and microsatellite will therefore be scored as homozygotes, so heterozygosity will be underestimated. Null alleles are a problem for heterozygosity-based estimators of genetic divergence, such as F_{ST} and R_{ST}, so it would have been a serious concern for this study if heterozygosity were hugely variable among loci.

The nature of the genetic structure was explored by fitting the relationship between ($\log_{10}$) geographic distance and population divergence (pairwise F_{ST} and R_{ST}). Significant isolation-by-distance (IBD) was found and when the cross-river sample pairs were excluded, the R^2 and slopes of these relationships were increased and *P*-values became more significant. This implies that geographical distance across the river and geographical distance along the river are not equivalent barriers to dispersal and gene flow, which is probably because the flightless *C. graminis* is unlikely to cross the river. Inspection of Figure 6.3 shows that very close populations separated by the river are as differentiated as populations on the same bank that are outside dispersal range. However, distant populations across the river were not very differentiated, in contrast to what might be expected. It is possible that downstream flood dispersal may cause sufficient gene flow between distant cross-river populations to cause this, although this should also occur for populations on the same bank. Only one population on the west bank of the river was sampled so it is difficult to draw any solid conclusions from this. However, Keller *et al.* (2004) showed that roads bisecting a forest are sufficient barriers to dispersal to cause population structuring in the carabid beetle *Abax parallelepipedus*, so it is perhaps to be expected that a river would be a significant barrier for *C. graminis*.

In previous studies that have sought to demonstrate intra-specific IBD for chrysomelids, both significant (McDonald *et al.* 1985; Congdon *et al.* 1997; Knoll and Rowell-Rahier 1998; de Jong *et al.* 2001; Kim and Sappington 2005) and non-significant (Sakanoue and Fujiyama 1987; Knoll and Rowell-Rahier 1998) results have been reported. Peterson and Denno (1998) fitted IBD relationships using Nei's (1973) G_{ST} (equivalent to F_{ST}, but not able to take negative estimates) for datasets from 43 published allozyme studies of phytophagous insects. Significant IBD was found in nearly half of all studies, the strength of which varied with dispersal ability. The strongest IBD was found for moderately dispersive species (able to disperse 1-20 km per generation), while highly dispersive species exhibited weaker IBD because gene flow operated over wider scales and prevented population divergence. However, sedentary species (dispersing <1 km) also showed relatively weak IBD, which was interpreted as being caused by an absence of gene flow across the wide scales covered in their analysis (>10 km). IBD relationships were unaffected by insect order, diet breadth or the number of allozyme loci scored, but were less likely to be evident when fewer than 10 populations were sampled.

In this study, significant IBD was found for a sedentary species using just eight populations (seven when the opposite bank population was excluded). This is probably because sampling was designed to range between populations likely to be linked by gene flow (<1 km apart) up to those that will be effectively isolated (~8 km apart). Thus the IBD that was missed for sedentary species in Peterson and Denno's (1998) review at wide geographic scales was detected across the scale of this study. The IBD relationships observed here suffered much scatter, but the along-bank R^2 values of 0.226 for F_{ST} and 0.388 for R_{ST} are typical of those reviewed by Peterson and Denno (1998) (a range of 0.00-0.64).

The key assumptions of IBD are that populations are equally outbred and at equilibrium (Rousset 1997). While comparison of within-sample F_{IS} values (Table 6.5) suggests that levels of inbreeding among the sampled populations were similar (although this may be confounded by any within-sample structuring) it is unlikely that *C. graminis* populations are at equilibrium given the turnover of tansy and population and range fluctuations that have been observed (Chapter 3). Nevertheless, IBD may be more robust to violations of its assumptions in linear habitats such as riverbanks than in two-dimensional systems because the geographical distance between sites may be more representative of the level of gene flow than in two-dimensional habitats where many alternative dispersal pathways exist. In these situations, IBD may perform as well as more complicated alternatives that account for the 'resistance' in movement between sites (McRae 2006).

Randomisation tests showed that for pairs of *C. graminis* populations on the same side of the river, those just outside the beetle's demographically established dispersal range (1 km) and those far beyond this range were equally diverged, while both were significantly more differentiated than populations within dispersal range. This is consistent with Peterson and Denno's (1998) finding of weak IBD for sedentary insects above their dispersal range but surprising since although there may be little beetle dispersal at any distance above 1 km, gene flow over multiple generations should cause the more separated populations to be more differentiated. A possible explanation is that this is an artefact of the small number of sampled populations and relatively low genetic diversity. In these circumstances F_{ST} cannot vary over the full extent between 0 and 1 (Petit *et al.* 1995) so there may be a maximum limit to estimated population differentiation. Another possibility is that long-distance dispersal events (possibly caused by flooding) are equally common over scales between 1 and 8 km such that levels of gene flow between these populations are equivalent.

6.4.3 Conclusions

This preliminary study has developed seven polymorphic loci (four allozymes and three microsatellites) that can be used to investigate population genetic processes in *C. graminis*. Using the microsatellite loci, I demonstrated that the tansy beetle population around York is significantly genetically structured and exhibits isolation-by-distance. Genetic divergence appears to plateau to a maximum once the demographically established upper dispersal limit of 1 km is exceeded. There was evidence of the river forming a barrier to dispersal, since the inclusion of cross-river samples weakened the IBD and close populations separated by the river were highly differentiated.

Chapter 7: Thesis discussion

7.1 Summary of findings

7.1.1 Oviposition ecology

Tansy beetle oviposition strategies and their consequences were investigated in Chapter 2. Most *Chrysolina graminis* eggs were found attached to non-host plant species in and around the periphery of tansy patches. There was circumstantial evidence that oviposition on non-host species may reduce the risk of cannibalism by adult beetles, since egg batches collected from matrix vegetation and those collected from patches with low adult densities hatched before those collected from tansy or high-density patches. This implies that eggs on tansy in high-density patches were on average younger than those from the matrix or low-density patches as a result of an increased risk of cannibalism. The beetle's oviposition strategy appeared to be fixed rather than condition-dependent, since the sizes of clutches laid on tansy or in the matrix did not differ and the proportion of clutches oviposited in the matrix was unaffected by adult density.

A consequence of this strategy is that the majority of larvae will hatch onto unsuitable vegetation and must move to find food, which presumably imposes a cost on them. It was demonstrated in Chapter 4 that larvae are unable to detect and move towards tansy from even very short distances so it is likely that many will die before they ever reach their food plant. However, post-hatching larval survival in the absence of tansy was surprisingly high and suggested that they have 4-7 days to find food before starvation. This window of opportunity will doubtless be lengthened by energetic gains from the cannibalism of sibling eggs after hatching, which was shown to occur in the laboratory. However, larvae hatching on tansy were just as likely to cannibalise their siblings as those hatching on matrix vegetation; again implying a fixed strategy.

7.1.2 Tansy beetle distribution

Globally, *C. graminis* appears to be in decline and this has certainly been the case in Britain over the last century. Populations have apparently disappeared from fenland areas in East Anglia, such as Wicken Fen, and the range at its final stronghold on the River Ouse has contracted. However, over the last decade the extent of the York population has been regionally stable, albeit with local fluctuations over a few kilometres. Part of the research presented in Chapter 3 involved setting up translocations that expanded the beetle's British range on the Ouse up to the northern-most location recorded last century. As these populations grow and spread out it is anticipated that the historical decline around York will have been reversed.

On the Ouse, the beetle occupies a network of patchily-distributed riparian tansy patches. Given this network structure, patches occupied by beetles are aggregated at spatial scales meaningful to beetle dispersal (up to 2.7 km). The upper dispersal limit recorded in Chapter 5 was 856 m, approximately a third of the scale of beetle aggregation. This implies that either there are unrecorded longer distance dispersal events common enough to ensure colonisation of patches 2.7 km apart or that this autocorrelation is built up through multiple dispersal events within and between generations.

Tansy patch occupancy by *C. graminis* is promoted by increased patch size (both in basal areas and maximum heights) and population connectivity (the distance to other beetles on the same side of the river, scaled by dispersal ability). This was confirmed by the expansion of beetles following translocations, whereby newly colonised patches were larger and of higher population connectivity than those remaining vacant. These results are probably caused by a combination of factors. Firstly it was shown in Chapter 5 that dispersal is spatially localised and targeted towards large patches. Emigration rates are higher in small patches with high perimeter-to-area ratios, which will mean that beetles have shorter residency times in smaller patches. Also, larger patches have higher carrying capacities so will be less vulnerable to stochastic extinction. Despite the clear relationships with patch size and isolation, there was little evidence for a role of patch quality in beetle occupancy, suggesting that tansy foliage from plants within the beetle's current range are of similar nutritional value with respect to the habitat quality variables measured in Chapter 3.

The network connectivities (the dispersal-scaled distances to other patches, multiplied by their areas) of occupied and unoccupied patches were similar, suggesting that beetles are not aggregated on the parts of the riverbank with the most clumped arrangements of tansy patches. This implies that beetles are unable to achieve an equilibrium distribution, as their dispersal patterns should mean they accumulate in these areas. The likely cause of this is turnover in the tansy patch network combined with gaps in the patch network and other barriers to dispersal. Thus there will be a lag between the current distribution of tansy and that of *C. graminis* (determined by the historical patch network and beetle distribution). The lag may be increased if beetle herbivory feeds back onto the spatial dynamics of tansy. For example, if tansy fitness were higher in the absence of herbivory then clusters of patches with high network connectivities would develop in areas where the beetle was currently absent.

7.1.3 Movement at different scales

C. graminis appears to use only one sense when navigating – contact chemoreception. No evidence of attraction to tansy or conspecifics was found but adult and larval beetles reduced their net displacements on tansy foliage. Adults achieved this by reducing the duration and frequency of movement, taking smaller steps and widening the range of turning angles between consecutive steps such that net displacements were reduced. In the matrix, adults switched to straighter and faster movement paths leading to increased net displacement and efficient location of patches. A random walk model based on these behaviours was able to explain the major trends in daily movements assessed with two mark-resight (MR) studies, provided appropriate patch boundary strengths were enforced in the model. Weak boundaries were optimal for one MR study, carried out early in the field season, while strong boundaries were required for the MR study carried out later in the season. A possible explanation for this is that tansy had grown by the time of the later MR study and emerged from the matrix vegetation. This would restrict emigration (strengthen boundaries) since beetles could not reach the matrix from the emergent patch edge.

Beetle dispersal was considered in Chapter 5, using a combination of a long-term (from *C. graminis*' point of view) multi-patch mark-recapture study and mathematical modelling. The mark-recapture data revealed female-biased and localised dispersal that was not biased up or down the river. Dispersal was more common during flooding, with inundation forcing beetles onto the flood embankment. Local dispersal was thus shown to be a key mechanism by which adult beetles persist through summer floods.

A general model of inter-patch dispersal was developed and parameterised from the mark-recapture data. In the model, patches competed for beetles based on their size and distance from the beetle's current location scaled by a dispersal kernel (a function describing the distance-dependence of dispersal ability). Emigration could either be modelled implicitly (emigration achieved when the source patch failed to hold on to its beetles) or explicitly (emigration was a function of patch size). The implicit model was selected as the most appropriate for *C. graminis* because, based on its movement behaviour, all beetles were likely to leave their patch over the timestep of the mark-recapture data. The model revealed that dispersal kernels with fat-tails (increased representation of long-distance dispersal) were the best fitting for *C. graminis* and produced realistic dispersal patterns. This suggested that females were slightly more dispersive than males and that dispersal was strongly biased towards large patches and away from small patches.

Thus, beetle movement was investigated at three scales – single movements, daily net displacements and fortnightly dispersal, allowing examination of the scale-dependence of these results. The relationships between individual movement behaviour and daily movements, as revealed by scaling up the random walk model, have already been considered. It would have been interesting to perform a similar study attempting to scale the random walk model up to the fortnightly dispersal movements. However, representing the 2 km of bank in which dispersal was quantified at the scale of the model's current landscape maps (1 cm^2 grid cells) would be excessively computationally demanding. Reducing the precision of the maps by coarsening their resolution would mean losing crucial information on the geometry of the patches, which would influence model-predicted emigration rates. Also, extra complications such as increased movement speeds induced by hunger (Chapter 4) may come into play that would prevent scaling to dispersal. While this may not have been of much importance for reproducing daily movements because most individuals were not outside patches for long enough to become hungry, this would almost certainly not be the case for fortnightly time periods. It also seems unlikely that the simple random walk model would have produced the observed long-distance dispersal events without the inclusion of heterogeneous behaviour (Skalski and Gilliam 2003) of which varying hunger-levels may be just one cause.

Sex appeared to have scale-dependent effects on movement. Individual movements and those at daily scales were largely unaffected by sex, while over fortnightly timescales, females were observed to move between patches slightly more often than males. It is possible that this is an artefact of the greater sample sizes recorded in the dispersal study, especially since the fitted dispersal kernels of males and females were only slightly different, implying they have very similar dispersal abilities. However, if this were a true effect then some factor not recorded at small and medium scales must influence movement decisions at larger scales to either speed up females or slow down males. Oviposition is an obvious factor that may influence females, but is unlikely to be the cause since the dispersal model suggested no inherent seasonality to dispersal while oviposition is highly seasonal. A possible non-seasonal explanation is that hunger has more of an accelerating influence on females than males, though no evidence of this exists.

Small patch area promoted emigration at daily and fortnightly timescales implying qualitative scale-independence. This would result from relatively greater perimeter-to-area ratios under any random walk model (Englund and Hambäck 2004). On the other hand, the height-dependent boundary permeabilities suggested by the results of the random walk model imply that wider-scale dispersal rates should vary with time as patches and the matrix vegetation grow and senesce through the season. This is not necessarily the case however, since even with emergent and resistant patch boundaries it may be that over two weeks beetle movements are sufficient to cause complete departure from all patches, so these small-scale spatial differences may be of minor importance when integrated over longer temporal periods.

Local tansy patch network structures had a bearing on movement at both daily and fortnightly scales. This was evident in the different movement patterns recorded in the two daily mark-resight studies, which the random walk model suggested were caused by the different arrangements, sizes and boundary permeabilities of the patches. In the dispersal model, achieved dispersal rates and distances are a product of the local patch network structure. An example of this is the model's ability to explain increased dispersal rates when flooding and mowing temporarily alter the patch network.

Qualitative scale-independence in the distribution parameters of movement distances was also evident. At all scales except for individual movements on matrix vegetation, net displacements were highly leptokurtic and right-skewed (Table 7.1) meaning that the presence of a minority of long-distance movements was conserved across scales. In reality, matrix step lengths are probably more leptokurtic and right-skewed than was recorded because beetles move faster in the matrix when deprived of food while the beetles observed moving in the matrix probably weren't hungry enough for this. This may also explain why the leptokurtosis and right-skew of inter-patch movements increase dramatically with scale (Table 7.1), as hunger will play a greater role over longer time scales and in the matrix.

Therefore, movement patterns observed at one scale were generally reflected in studies at different scales. The main exception to this is that sex-biased dispersal was observed in mark-recapture data and suggested by the dispersal model, while daily and individual movements of males and females were similar. However, the sex difference in dispersal was slight and based on greater sample sizes, which may explain this.

Table 7.1. The kurtosis and skewness in displacements made by adult tansy beetles over different scales. Individual steps were those recorded in the movement path experiments of Chapter 4. Daily intra and inter-patch movements are combined from the mark-resight studies at Skelton in 2003 and 2004 (Chapter 4). Fortnightly dispersal distances were recorded with mark-recapture at Clifton in 2004 and 2005 (Chapter 5) and combine data from males and females.

Movement scale and type	**N**	**Kurtosis**	**Skewness**
Individual steps on tansy	193	7.82	2.37
Daily intra-patch movements	515	10.09	2.28
Individual steps on matrix vegetation	107	0.23	0.92
Daily inter-patch movements	79	5.97	2.24
Fortnightly dispersal events	131	19.26	4.31

7.1.4 Genetic structure in relation to dispersal

Using three independently inherited and polymorphic microsatellite loci, significant population structure and deviations from Hardy-Weinberg equilibrium were identified among eight *C. graminis* subpopulations. Significant isolation-by-distance (IBD) was evident and the strength of the relationship was increased when it was solely based on data from populations on the same side of the Ouse. Randomisation tests suggested that for populations on the same bank, divergence increased with geographic distance until the maximum dispersal range of *C. graminis* (estimated in Chapter 5 as ~1 km) was exceeded and then remained constant. In theory just one migrant per generation is sufficient to prevent differentiation (Allendorf 1987) so this result appears to validate the demographically established upper dispersal limit. The genetic data also provided some evidence towards the river being a barrier to dispersal, although only one population from the opposite bank to the other seven was sampled so it was difficult to draw conclusions from this despite the overwhelming likelihood that beetles find it difficult to cross the river.

7.2 The future for the tansy beetle

The most serious threat to the persistence of *C. graminis* around York is probably degradation of the riparian tansy patch network through over-grazing and development. These will create large gaps between clusters of tansy patches, which beetles will find it hard to cross. Colonisation after local extinctions will thus be prevented or delayed to the point where regional extinction will become more likely. To prevent this from occurring, appropriate bank management may involve annual mowing when beetles are pupating or overwintering and leaving unmown refuge patches. Other specific conservation measures may include translocating beetles, planting tansy patches or including tansy in seed mixes for new flood embankments. The success of sowing tansy seed after embankment reconstruction has been shown by the huge population now found at South Ings. Tansy seed used in this way should be sourced locally to ensure that different tansy chemotypes (plants with different compositions of defensive chemicals, see Chapter 3), which are known to vary geographically on national scales, are not sown. Beetles may be poorly adapted to the chemical defences of tansy from other parts of the country, which may reduce the efficacy of this conservation measure.

It seems that *C. graminis* should survive around York for the foreseeable future provided that managers are aware of its status and any management is sensitive to the beetle's phenology and life cycle. However, since the beetles' British range is very small and the species has weak dispersal ability it will be potentially susceptible to regional processes that degrade the suitability of the entire Ouse. Catastrophes such as severe summer flooding operate over the entire range of the species so extremely severe events such as this could cause extinction. Climate change may pose a more chronic threat to *C. graminis* if it alters the riparian landscape around York rendering it less suitable. For example, as the climate warms summer flooding of the Ouse may become more common (Longfield and Macklin 1999) or tansy populations may decline, with knock-on effects for the beetle. While other insects track climate change by shifting their ranges (Parmesan *et al.* 1999), it seems unlikely that *C. graminis* will be able to do so without intervention because of its sedentary nature and regionally fragmented habitat.

A further chronic threat to *C. graminis* is the advance of the invasive Himalayan balsam *Impatiens glandulifera*. This species reduces the vegetation diversity of British riverbanks by both shading out other plants and outcompeting them for the attention of pollinators (Chittka and Schürkens 2001; Hulme and Bremner 2006). In many sections of the riverbank Himalayan balsam has established dense stands of vegetation from which tansy and other native vegetation are apparently displaced. Ideally *I. glandulifera* would be controlled as part of the conservation strategy for *C. graminis*, but this species has proved difficult to control in the past (Wadsworth *et al.* 2000) so this may be impractical.

7.3 Further research

Many questions regarding the spatial ecology of *C. graminis* have been answered in the course of the research presented in this thesis. However, there remains, as always, much scope for further work. Some suggestions for this are given below.

- Further investigation is required into the balance between the density-dependent risks of cannibalism for eggs laid on and off tansy and the cost to larvae hatching in the matrix. These should be investigated with garden experiments involving the translocation of eggs onto or near potted plants with different adult densities and monitoring the fate of eggs and newly hatched larvae. The extent to which the beetle's observed oviposition strategy is adaptive would thus be assessed.

- Further research into *C. graminis* movement behaviour should attempt to record multiple movement paths of the same individuals over longer timescales and under different levels of food deprivation. These data could be used to fit a pre-defined correlated-random walk model with step lengths and turning angles being functions of habitat, hunger and other factors. If sufficiently long paths of many individuals are recorded, a meta-analysis among individuals can be performed using a Bayesian state-space approach that fits movement parameters to each individual, themselves drawn from a specified distribution (Jonsen *et al.* 2003). This would allow a mechanistic understanding and quantification of the heterogeneity in movement behaviour that is known to be so important for long-distance dispersal (Skalski and Gilliam 2003).

- It is easy to envisage the development of a simple individual-based tansy beetle model from the results of this thesis. The dispersal model (Chapter 5) could be used to move beetles around a landscape of tansy patches, with reproduction on each patch defined by a function of the total number of female beetles within the patch through the season. This model could be used to investigate the likely success of translocation schemes, or to evaluate the effects of changing bank management on the beetle. However, realistic predictions from such a model would depend on a realistic model of the spatial dynamics of tansy, as dispersal is highly dependent on the local landscape structure. Thus, further work into tansy growth and turnover and their interactions with beetle occupancy are required.

- The dataset on microsatellite frequencies collected in thesis could be expanded by sampling more populations, particularly increasing the spatial range of the sampling and including more populations on the west bank. Allozyme data should also be collected. A more thorough examination of genetic isolation-by-distance, including a test of the significance of barriers presented by the river and other landscape features such as woods or buildings should then be carried out by performing a partial Mantel test, which is similar to analyses of covariance (Legendre and Legendre 1998; Castellano and Balletto 2002). If further microsatellite markers are developed, individual-based multilocus genetic analyses such as population assignment methods (Wilson and Rannala 2003) could be used to investigate the effects of distance and dispersal-barriers on gene flow and parentage analyses could be used to investigate the beetle's mating system including sperm precedence and multiple mating (Beebee and Rowe 2004).

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