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Ecological and genetic effects of urbanization and fragmentation on carabid beetles from Belgian beech forests

Ecologische en genetische effecten van urbanisatie en fragmentatie op loopkevers in Belgische beukenbossen

door

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In memoriam of Konjev

Table of contents

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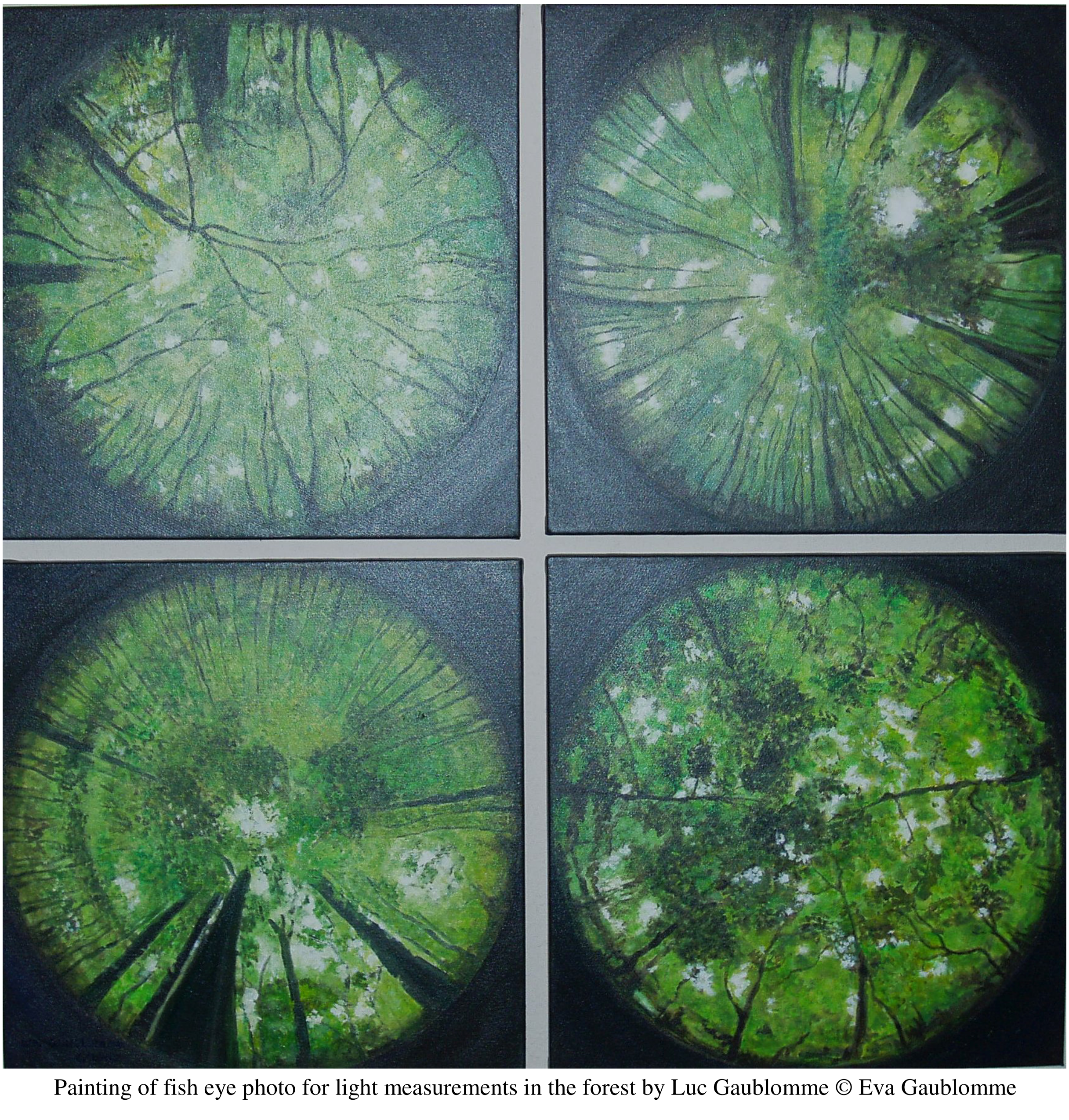

Painting of fish eye photo for light measurements in the forest by Luc Gaublomme © Eva Gaublomme

Chapter 1

INTRODUCTION AND THESIS OUTLINE

Chapter 1

Introduction and thesis outline

1.1 Area and isolation effects as a consequence of habitat fragmentation

Landscape modification for agriculture and urbanization puts severe stress on natural ecosystems because of increasing habitat loss and deteriorating habitat quality. Forest fragmentation is an important process contributing to the present-day concern over the loss of biodiversity and rates of species extinction (Meffe & Carroll 1997, Fahrig 2003, Ewers & Didham 2006). It results in a landscape composed of habitat fragments (e.g. forest) with something else (the matrix) between the fragments. This results in a decrease of population size and increased isolation of habitat patches (MacArthur & Wilson 1967). Besides the direct reduction in population size, small populations become more prone to extinction as stochastic variation may result in a higher probability of extinction in small compared to large populations. The assumed processes likely to be involved in species extinctions as consequences of habitat fragmentation have been conceptualized in the so-called extinction vortex (Frankham et al. 2002, Gilpin & Soulé 1986).

The higher extinction probability due to stochastic effects can be of demographic, environmental as well as genetic kind. Demographic stochasticity results from random fluctuations in population sizes due to intrinsic year-to-year variation. Their effects on local extinction are expected to be proportionally much larger in small populations. Environmental stochasticity influences populations regardless of their size, but smaller habitat patches have less buffer capacity or variation in microclimates to escape from the negative impacts of environmental stress. Genetic stochasticity are random fluctuations in allele properties, with expected profound effects on the stability of populations (see further 1.3).

Research on habitat fragmentation was often based on the equilibrium theory of island biography (MacArthur & Wilson 1967) and the theory of metapopulation dynamics (Collinge 1996, Baguette 2004). The first theory predicts that the number of species on an island (habitat fragment) is a function of island size and distance to the mainland (continuous unfragmented habitat) so that smaller, more isolated fragments retain fewer species than less isolated ones. The second theory states that subpopulations created by habitat fragmentation may depend more strongly on immigration for their persistence than populations from unfragmented habitat. If the immigration rate is however insufficient to counteract the effect of local extinction, the metapopulation may become extinct (Hanski 1994). The structure of the surrounding matrix (Stow et al. 2001) and the dispersal potential of the species (Driscoll 2004) can influence immigration among patches within fragmented landscapes. However, these theories had some shortcomings in their applicability to fragmentation, which can be due to the boundaries of habitat fragments (habitat edges) that can also profoundly influence ecological patterns and processes (Gascon & Lovejoy 1998), and because the surrounding matrix was previously considered as ecologically irrelevant (McIntyre & Hobbs 1999, Murphy & Lovett-Doust 2004, Kupfer et al. 2006). Nowadays it is recognized that also edge effects and matrix type play an important role, especially in human-dominated landscapes (Ricketts 2001, Laurance 2008, Prevedello & Vieira 2010, Öckinger et al. 2012).

1.2 Edge effects and matrix effects in an urban context

Urbanization modifies the original habitat through different forms of anthropogenic land use (Vitousek et al. 1997) and turns urban ecosystems into spatially heterogenic and temporally dynamic regions which differ fundamentally from their original environment (McIntyre 2000). Insects in urban areas are at risk primarily due to loss of habitat, but changes in habitat quality can result in either declines or increases in the abundance of insects (Connor et al. 2002). A matrix hostile to dispersal has been shown to hinder movement between patches (Ricketts 2001, Baum et al. 2004), thus a matrix of urban land-use may be a greater obstacle for dispersal than a matrix of rural land-uses, if animals are less likely to move through an urban matrix. Besides direct effects of urbanization, fragmentation also leads to an increase in heterogeneity in habitat quality because the fragments may undergo a change either directly (through conversion) or indirectly (through edge effects) (Murcia 1995). Edge effects refer to changes in biological and physical conditions that occur at a patch boundary and within adjacent patches (Murcia 1995, Lindenmayer et al. 2008). They are thus the result of the interaction between two adjacent habitats, in our context the forested area and the non-forested area (the matrix), and result in a habitat that is distinct from both main habitat types. Forest edges are easily invaded by species from the surrounding, human-altered matrix and are supposed to have a negative impact on the forest organisms that remain in the fragments. Although, some studies note that edge communities can consist of a few specialized species, so called edge preferring species (i.e. species being dependant on the edge habitat during a certain time of their life cycle) (Didham 1997, Magura et al. 2001, Molnar et al. 2001, Lövei et al. 2006), edges are generally expected to have detrimental effects on species, specialized on the interior habitat. It is intuitive that when the size of habitat patches decreases, the ratio of edge to interior habitat increases proportionally resulting in an increase in edge effects (Saunders et al. 1991, Fagan et al. 1999, Ries et al. 2004). Moreover, in small habitat patches there may be no interior habitat left (Laurance & Yensen 1991, Tscharntke et al. 2002, Ewers & Didham 2006) and generalist species increase due to the contribution of the surrounding landscape matrix. While both habitat area and edge may influence individuals, populations and communities, it is crucial to understand their relative role for implementing conservation strategies (Fletcher et al. 2007). Little clear evidence currently exists for area and edge effects interacting in fragmented landscapes (Nour et al. 1993, Didham et al. 1998, Matthews et al. 1999, Barbosa & Marquet 2002, Galetti et al. 2003, Fletcher 2005, Ewers et al. 2007). Examination of both area and edge effects and their interaction are therefore the best option to understand their contribution to habitat fragmentation (Fletcher et al. 2007).

1.3 Population genetic consequences of habitat fragmentation

Population genetics is the study of allele frequency distribution and change under the influence of the four main evolutionary processes: natural selection, genetic drift, mutation and gene flow. It concerns the genetic constitution of populations and how this constitution changes with time. Genetic diversity has become one of the keywords of scientists who are concerned about sustainable management of forests. Behind this concern is the assumption that high levels of genetic diversity provide a guarantee for the survival and persistence of species that are strictly bound to forest habitat. Knowledge of the genetic structure within and among populations, combined with ecological data and the spatial configuration between habitats can provide a first step in assessing the extent to which species have reacted to the severe loss of habitat experienced during the last century.

Habitat fragmentation is leading to a reduced size and increased isolation of habitat patches (Wright 1931) and as a consequence resulting in a lower population size and a higher isolation degree of the remaining local populations (MacArthur & Wilson 1967). Small populations, occurring in isolated habitat fragments, face threats from increased inbreeding (short-term genetic effect), further loss of genetic variability due to genetic drift and consequently lowered evolvability (long-term genetic effect of fragmentation) and random fixation of deleterious alleles (Frankham et al. 2002). Inbreeding increases the chance that deleterious alleles are combined within single individuals, leading to lowered reproduction and reduced survival (inbreeding depression) of a larger proportion of the population. Random genetic drift (genetic stochasticity) leads to further loss of genetic diversity (genetic erosion). Especially in the absence of immigration, and thus gene flow between populations, lost genetic variation is not complemented by immigrants and genetic impoverishment renders the populations of a species less evolvable and therefore again more vulnerable.

1.4 Forest history in Belgium

Western European forests experienced a long and severe fragmentation in several waves starting with the Roman period. A first regression in forest area took place until the end of the Roman period (around 1000 A.D.), followed by a partial forest recovery during early Medieval period. There was renewed deforestation from the late Middle Ages onwards, followed by a large-scale deforestation in the 19th century (Tack et al. 1993, Verhulst 1995).

In Belgium, as in many other parts of Europe, forests have been under human pressure for about 7000 years. Their history in general is one of woodland destruction, fragmentation and degradation, although locally interrupted with periods of forest rehabilitation and expansion (e.g. in Flanders between y1300-1800). Around y1850, woodland area reached its absolute minimum in this region, with subsequent reforestation occurring with pine and poplar stands that are generally assumed to be less valuable from a nature conservation perspective (Tack et al. 1993). The decrease of ancient forests has thus been accompanied by a serious decline in forest habitat quality. Forests nowadays cover about 20% of Belgium, but there are large differences between regions, with less than 10% of area forested in Flanders (Hermy et al. 2003). The proportion of these forests that can be defined as ‘ancient’, i.e. forest that persisted during the past 230 years (i.e. since the first systematic maps of de Ferraris, y1770-1778 appeared), is however less than 23.000 ha or 2 % of the total area (De Keersmaeker et al. 2001).

Recent investigations have revealed that evolution often occurs on contemporary time scales, often within decades and associated with habitat loss and degradation, and is therefore highly relevant to many conservation situations (Stockwell et al. 2003). The ecological forest history of the past hundreds of years is thus expected to be still visible in the present-day plant and animal assemblages, their species composition, and morphological and genetic constitution. Gurdebeke et al. (2003) formerly studied the genetic diversity and differentiation of a spider in this region. However, we have barely scratched the surface of documenting and understanding the patterns and processes in diversity and evolution of organisms that accompanied the ecological history of our forests. There is thus an urgent need to study, protect and, if possible, rehabilitate these ancient forests.

1.5 Why studying ground beetles for forest conservation purposes?

Beetles (Coleoptera) are one of the most biodiverse animal groups. Among these, ground beetles (Carabidae) are one of the most speciose families with approximately 35000 species (Lorenz 2005), of which 404 species in Belgium (Desender et al. 2008). Apart from their high diversity, they possess several properties that make them very suitable as indicators to assess the effects of habitat fragmentation and deterioration (i) their taxonomy is stable and both identification and sampling are relatively easy (ii) for most species, the ecology and habitat preference is very well documented in Belgium and elsewhere in Europe (Desender et al. 1994, Turin 2000) (iii) the historic and present geographic distribution of these beetles is very well documented for Belgium, and even led to one of the first compiled Red List of a species group in Flanders (Desender et al. 1994, Desender et al. 2008) (iv) Moreover, carabid beetles are one of the very few invertebrate groups where archaeological data are available (Desender et al. 1999, Ervynck et al. 1994). Hence, given that many species are habitat-specific and considered good ecological indicators because they are sensitive to human-induced disturbances (Rainio & Niemelä 2003, Pearce & Venier 2006). The generation time, as for other arthropods, is short making them ideal to study the relationship between habitat fragmentation and population genetic structure (Keyghobadi 2007).

Habitat affinity groups

An important classification of species into functional groups is the degree of specialization of different species. It appears that more-specialized species may be (directly or indirectly) more susceptible to habitat deterioration and fragmentation than generalist species, because, by definition, they are more closely linked to a particular host-, prey- or habitat-type and have little flexibility to cope with a changing environment (Didham et al. 1996, Magura et al. 2004, Sadler et al. 2006) .

With respect to the current study, species were divided into four groups as follows: (i) forest stenotopic or forest specialist species were defined as species occurring exclusively in permanent woodlands. Several stenotopic woodland species appear to be linked to ancient forest (Assmann 1999). Ground beetle species belonging to this group almost universally have a very limited dispersal power (Assmann & Gunther 2000), and are mostly wingless (Desender et al. 1999), hampering colonisation between isolated forests. Typical species belonging to this group are *Carabus auronitens*, *C. problematicus*, *Abax ovalis*, *Cychrus attenuatus* and *Pterostichus cristatus*. (ii) Forest eurytopic or forest generalist species were defined as those that frequently or predominantly occur in woodlands but are also found in other, non-wooded habitats. (iii) The third group eurytopic or generalist species were defined as species that do not show a clear habitat preference and occur in a wide range of habitats varying from woodland to open landscape like arable field. (iv) A last group comprises open landscape species, defined as species mainly occurring in open landscapes like arable field, meadows,… Data on the distribution of each species were derived from the Red List and Atlas (Desender et al. 1994, Desender et al. 2008a, b).

Dispersal capacity

Understanding how species persist within and disperse across fragmented landscapes is essential for effective landscape management (Rykken et al. 2011). A most suitable characteristic of using carabid beetles in conservation research is their enormous inter- and intraspecific variation in dispersal capacity. This is reflected in differences in wing size and flight muscle development and as such easily measurable under field conditions. Indeed, research in carabid beetles appeared pivotal to test theoretical hypotheses on dispersal evolution (Den Boer et al. 1980). In general, three different dispersal classes are discerned i.e. (i) species are constantly winged, (ii) species that show a wing dimorphism or polymorphism and (iii) species that are constantly without wings (Den Boer et al. 1980, Desender et al. 1989). It is generally accepted that low dispersers are at a greater risk in a changing environment (Roff 1986), as their inability to disperse over long distances by flight forces them to cross unsuitable habitat if they move to patches that are not connected by interior habitat (Hanski 1998). This has been clearly demonstrated in a multitude of studies, where the response of brachypterous species towards fragmentation is generally much stronger compared to macropterous and wing di- and polymorphic species (De Vries et al. 1996, Rainio & Niemelä 2003, Yamashita et al. 2006, Hendrickx et al. 2009, Jelaska & Durbesic 2009). On the other hand macropterous species are able to disperse better, but there is a higher cost in terms of building the flight apparatus and in using it (Kotze & O'Hara 2003). Brachypterous species are in general both more fecund and reproduce earlier than macropterous species (Roff 1986), but this is not always holding (Aukema 1991).

1.6 Globenet

A global network (Niemelä et al. 2000) was set up to investigate the effects of urbanization on ground beetles in forest fragments.

The ultimate aim of the Globenet is to assess and compare the effects of urbanization on biodiversity in several countries around the world. In order to achieve this goal, a unified methodology and one group of organisms have been chosen: carabid assemblages of forests are sampled along urban-rural gradients. Such gradients represent a continuum of increasing human pressure and are subdivided into three classes: rural, suburban and urban. A disturbance gradient from urban through suburban to rural environment is an effective framework for studying the effects of urbanization on ecological systems (McDonnel & Pickett 1990, McDonnell et al. 1997, Niemelä et al. 2000). By comparing results from different cities, Globenet seeks to separate general, recurring effects on biodiversity from those that depend on local environments or particular biotic assemblages. This information can be useful in land use planning, depending whether the changes will affect biodiversity in similar ways across the globe or whether their impact will depend more on unique local conditions.

1.7 Study species

The population genetic studies in chapter 6 and 7 were performed on two forest inhabiting species, i.e. *Carabus problematicus* and *Carabus violaceus (*Fig 1).

Carabus problematicus

This carabid beetle occurs from Finland to Southern France, including the British Isles (Turin 2000), with Belgium situated in the centre of its distribution area. It is a strictly nocturnal beetle and an autumn breeder with larval hibernation and overwintering adults, thus having a two-year life cycle. It is feeding on snails and insects (Skuhravy 1959). The species is wingless, but movement up to 70-75 meters per night has been observed (Neumann 1971, Rijnsdorp 1980). In Belgium the species is restricted to forests, except in the Campine region where it is commonly found in heath land, although they do probably not reproduce in the heath land (Den Boer 1977). Therefore in the absence of heath land this species can be described as a forest specialist species.

Fig. 1 – Left Photo: *Carabus problematicus*, right Photo: *Carabus violaceus*.

Carabus violaceus violaceus

European species with an Atlantic distribution. It is strictly nocturnal. The newly emerged adults occur in summer and reproduce mainly in August. Some adults hibernate and enter upon a second breeding in the following summer (Lindroth 1985). It is feeding on snails, carrion and mushrooms. The species is wingless, but it is able to cover distances on the ground of more than 10m in 24h and a few hundred meters in longer time-spans (Thiele 1977). The species is commonly found in different types of forest, but preferably in humus-rich and older forests and can best be described as a forest generalist species. According to Blumenthal (1981) 4 km of open grassland would prevent the species from colonizing neighbouring forests and they avoid to cross paved roads (Mader 1984, Keller et al. 2004).

1.8 Study area

Sampling set up for ecological studies (chapter 2 and 3)

Flanders, the northern part of Belgium, as well as other regions in Western Europe, is scarcely covered with forests (8%). This region has a long history of intense agricultural exploitation and high population densities, which resulted in a complex cultural landscape with a dense urban and infrastructural network.

The Brussels Capital Region in Belgium has a surface area of 160 km^2 and a very high population density of around one million inhabitants. Further expansion of the region is a considerable threat to the remaining natural habitats (Gryseels 1998).

Our sampling was carried out in 13 transects distributed over 10 forests situated in the capital Brussels (50°74’-50°89’N, 4°29’-4°41’E) in Belgium (Fig 1). The sites selected for study have: (i) a similar soil type (loam), (ii) beech (*Fagus*) dominated stands, (iii) >230 years old (i.e. since the maps of de Ferraris, 1775) and (iv) no evidence of recent severe anthropogenic disturbances.

Sampling was performed in three different areas in the city. Four different transects were situated in the Soignes forest (4383 ha). This is the largest area of forest in the northern part of Belgium accounting for 60% of the Brussels public green areas. West of the Soignes forest we investigated six remnant forests that were once connected to the Soignes forest, also with old trees that, prior to its definitive protection (since 1842), were part of large private estates and were later incorporated into prosperous neighbourhoods of villas or large forested parks.

In the north of Brussels another three forest transects (Dielegembos, Poelbos, Laarbeekbos) were selected, originating from afforestation in the 18th century of old calcareous limestone quarries and therefore having a different history compared to the other studied forests (Fig 2 and 3, Appendix 1).

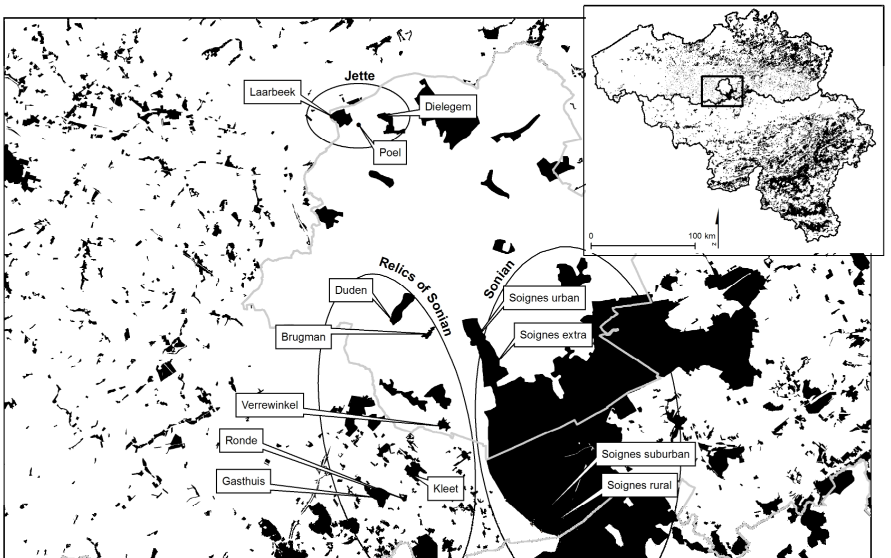

Fig. 2 – Sampling locations in and around Brussels, the capital of Belgium (in right upper frame). The dark areas represent forest. The three different sampling areas are encircled.

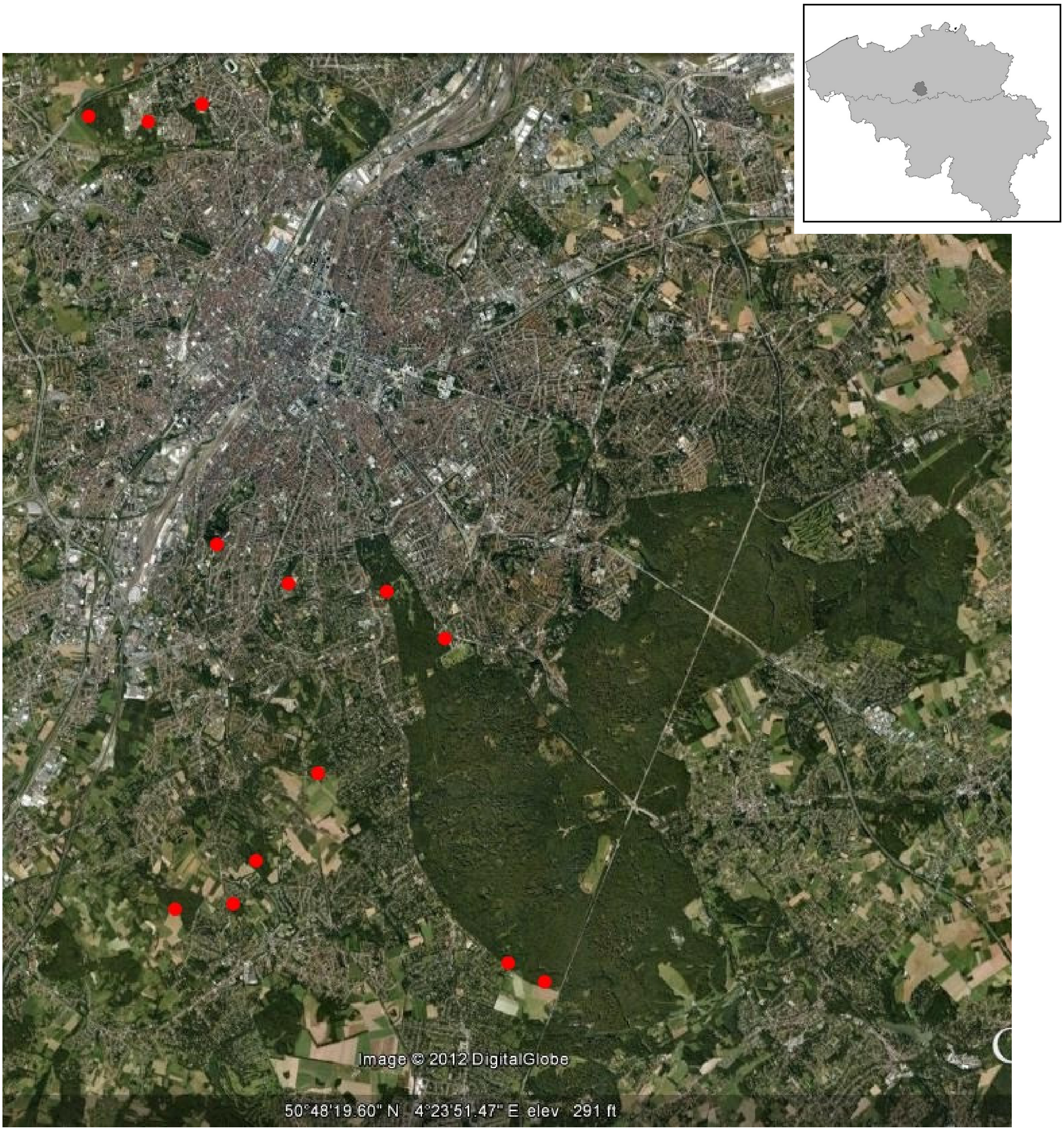

Fig. 3 - Aerial Photo showing the different forest fragments around the city of Brussels with the 13 sampled transects indicated as red dots. The dense urban character is clearly visible. The small inset map shows the location of the densely built-up area of Brussels in Belgium.

In each of the three areas we selected for a range of surrounding matrices going from completely surrounded by buildings or pavement and therefore highly influenced over a slight more semi-natural environment (i.e. garden, park,…) to a semi-natural environment (i.e.

arable field, meadow,…). All studied forests have an abrupt boundary between forest vegetation and surrounding matrix and fragment size ranging from 5.3 ha to 4383 ha. Beetles were sampled using pitfall traps (mouth diameter = 95mm, 90mm deep) and arranged in line transects at each site, running perpendicular from 30 m outside the forest (if possible), at the forest border (0 m), and at 30 m, 60 m and 100 m into the forest. A distance of 100 m into the forest was the maximum achievable distance due to size restrictions in the smallest fragment. At each distance from the forest edge, a plot of three pitfalls, parallel to the forest edge, and 5 m apart, were installed (Fig 4). In total, 174 pitfalls filled with a 4% formaldehyde solution to kill and preserve the collected arthropods were used. Traps were operational from 1 March 2002 until 19 November 2002 and emptied fortnightly. Beetles captured at each plot were pooled to obtain a single sample.

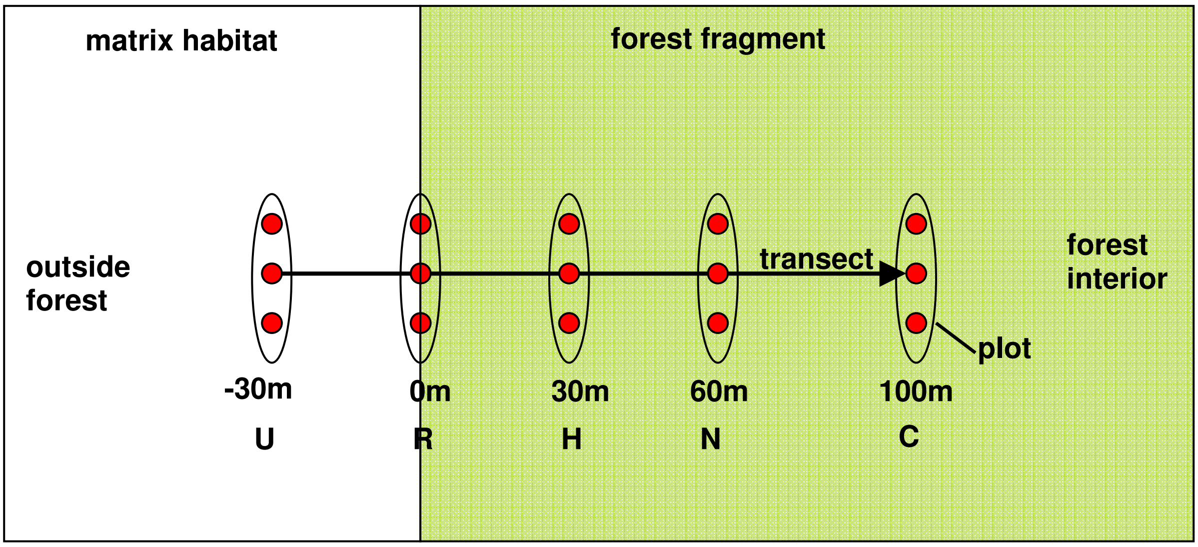

Fig. 4 - Schematic overview of sampling set up

Sampling set up for population genetic studies (chapter 6 and 7)

At each transect described above, six pitfalls (at about 5m from each other) were installed without fixative in order to obtain live beetles. The top (5 cm) of a plastic bottle (diameter = 10cm) was cut off and put inversely in the lower half of the bottle, functioning as a funnel. A roof is placed above these traps to protect against rain (Fig 5). The traps were emptied fortnightly over a period of six months (April until September 2002). The species needed for genetic research were frozen in separate tubes and stored at -80°C.

Fig. 5– left Photo: Gasthuis forest – right Photo: live beetle trap

In the forest fragments described above, *C. problematicus* was only present in three out of the thirteen sampling plots. Additional plots, scattered throughout Belgium, were sampled (Fig 6, Appendix 1), with a detailed sampling in the region of Brussels, where multiple plots where sampled within the different fragments (Appendix 1).

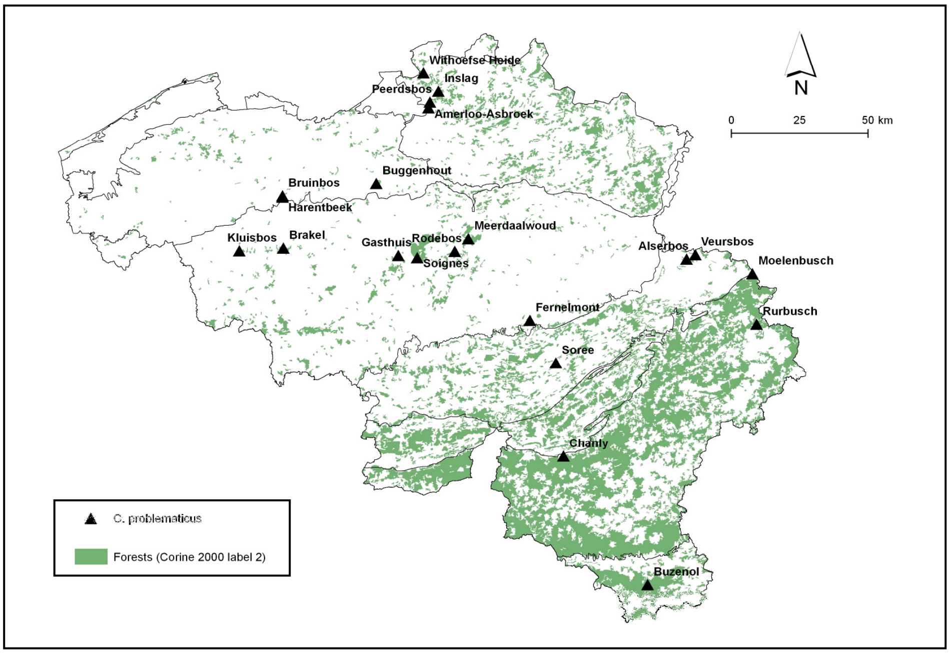

Fig. 6 – Geographic distribution of studied *C. problematicus* populations in Belgium. Forests are indicated in green and sampling locations with triangles.

The populations of *C. violaceus* were all sampled in the region of Brussels (Fig 7, Appendix 1). In total we investigated 19 sampling locations, of which eleven are located within the large contiguous Soignes forest. The other six fragments were once connected to the Soignes forest.

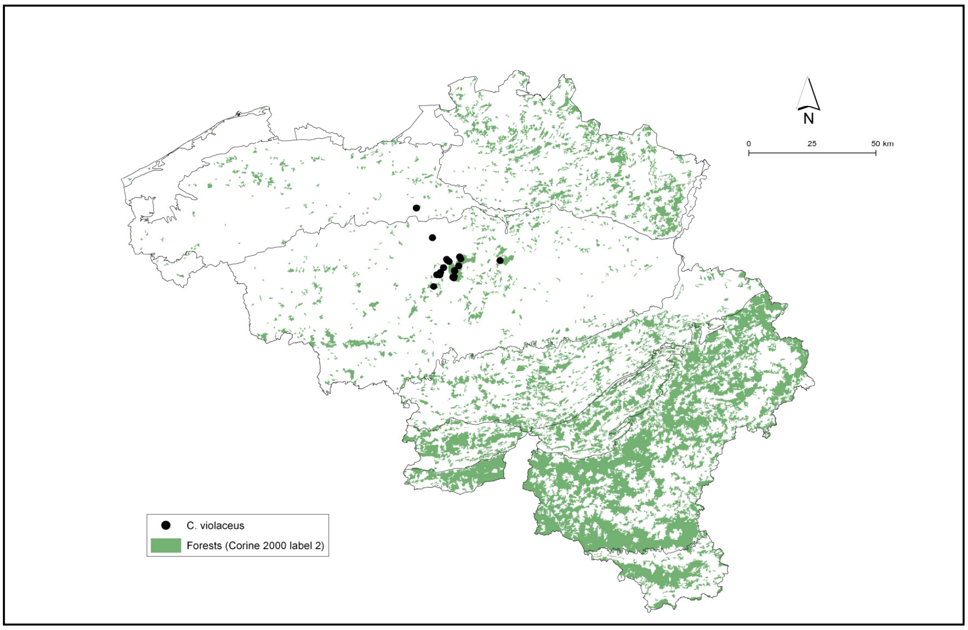

Fig. 7 - Geographic distribution of studied *C. violaceus* populations in Belgium. Forests are indicated in green and sampling locations with dots.

Sampling locations wherein both *C. violaceus* and *C. problematicus* are examined and compared are restricted to Buggenhout, Gasthuis, Meerdaal and two sampling plots in Soignes forest (Appendix 1).

1.9 Objectives and outline of the thesis

This thesis focuses on the response of carabid beetles towards fragmentation and urbanization in a highly modified urban landscape. Western European forests are a good example of a highly fragmented habitat. Belgium in particular, with its extremely dense human population in Flanders (around 6.2 million on a total area of 13500 km²), forms an excellent study area. Two important effects of fragmentation, patch size effects and edge effects (including matrix effects and area effects) are highlighted in this thesis. These effects are investigated on different levels: community level, habitat affinity group level and species level (chapter 2-3).

In a second part we focus on the genetic variation in different populations of two ground beetles with varying requirements to their environment. They both have a low dispersal capability and are therefore extremely vulnerable to the effects of habitat fragmentation (chapter 6-7). We also contributed in the development of highly variable genetic markers to assess the effects of fragmentation (chapter 4) and tested the influence of sampling on survival of two species (chapter 5).

The final goal of this thesis is to determine clear and specific guidelines for a better management of fragmented forests. There is a strong need for such guidelines especially within a densely populated area where forest fragments are only the remains of a rich past.

In **Chapter 2** we study the ground beetle species assemblages along the gradient from outside the forest to the forest interior with forests differing in size. We emphasize on the importance of area and edge effects on ground beetle communities and test for the inter relationship between these two effects.

An essential step in managing urban environments more effectively is a decent understanding of the interplay between landscape (matrix effects) and local factors (patch effects) that affect urban biodiversity. This part of the study was investigated in **chapter 3**.

In **chapter 4** we describe the development of microsatellite marker loci for the strict brachypterous forest species *Carabus problematicus*

Since some populations of ground beetles are at risk we compared the mortality rate of two different techniques of DNA sampling in *Carabidae* and tested their usage in allozyme electrophoresis and microsatellite screening in **chapter 5.**

Chapter 6 deals with the genetic structure of the forest specialist *C. problematicus* populations in a fragmented landscape and how this can be related to the factors forest area and geographical distance..

In **chapter 7** we describe the genetic structure of the forest generalist *C. violaceus* and compare the results with the forest specialist *C. problematicus*.

In **chapter 8** we summarize and discuss the main results and highlight topics that need further attention.

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1.11 Appendix

Appendix 1- Sampling locations and use of these locations in the different chapters.

	Sampling along edge gradient	Sampling *C. problematicus*	Sampling *C. violaceus*	Chapter
Alserbos		*X*		6
Amerloo-Asbroek		*X*		6
Brakel		*X*		6
Brugmanpark	X			2/3
Bruinbos		*X*		6
Buggenhout		*X*	*X*	6/7
Buzenol		*X*		6
Chanly		*X*		6
Dielegem park	X			2/3
Duden park	X			2/3
Fernelmont		*X*		6
Gasthuis forest	X	*X*	*X*	2/3/6/7
Haller forest			*X*	7
Harentbeek		*X*		6
Inslag		*X*		6
Kleet forest	X		*X*	2/3/7
Kluisbos		*X*		6
Laarbeek forest	X		*X*	2/3/7
Meerdaalwoud DR		*X*		6
Meerdaalwoud J+60		*X*		6
Meerdaalwoud J-40		*X*		6
Meerdaalwoud J-60		*X*		6
Meerdaalwoud KL		*X*	*X*	6/7
Meerdaalwoud O+60		*X*		6
Meerdaalwoud O-115		*X*		6
Moelnbusch		*X*		6
Peerdsbos		*X*		6
Poel forest	X			2/3
Rodebos		*X*		6
Rodebos vallei		*X*		6

	sampling along edge gradient	Sampling *C. problematicus*	Sampling *C. violaceus*	**Chapter**
Ronde forest	X		*X*	2/3/7
Ruhrbusch		*X*		6
Soignes A			*X*	7
Soignes Cam Cru			*X*	7
Soignes extra	X		*X*	2/3/7
Soignes F			*X*	7
Soignes KL			*X*	7
Soignes NV			*X*	7
Soignes P		*X*	*X*	6/7
Soignes RK		*X*	*X*	6/7
Soignes rural	X		*X*	2/3/6/7
Soignes suburban	X		*X*	2/3/6/7
Soignes urban	X		*X*	2/3/7
Soree		*X*		6
Verrewinkel forest	X		*X*	2/3/7
Veursbos		*X*		6
Withoefse heide		*X*		6

Top left: beech nuts, top right: view on Rondebos, bottom left: field work team, bottom right: pitfall trap © Eva Gaublomme

Chapter 2

THE EFFECTS OF FOREST PATCH SIZE AND MATRIX TYPE ON CHANGES IN CARABID BEETLE ASSEMBLAGES IN AN URBANIZED LANDSCAPE.

Chapter 2

The effects of forest patch size and matrix type on changes in carabid beetle assemblages in an urbanized landscape.

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2.1 Summary

In this study we compared ground beetles (Carabidae) from a range of different forest fragments along an urbanization gradient in Brussels, Belgium. We address the following questions: (i) How does the degree of urbanization in the surrounding habitat affect forest beetles, and does it interact with the effects of patch size and distance to forest edge? (ii) Do these factors have a different effect at the level of individual species, habitat affinity groups or total community? During 2002 we sampled 13 forest plots in 10 forest patches, ranging in size from 5.27 - 4383 ha. The beetles were captured using transects of pitfall traps from the edge to a distance of 100 m into each woodland and identified to species level. Effects of urbanization, forest size and forest edge were evaluated on total species number, abundance and habitat affinity groups and ten abundant, widespread model carabid species. Overall, the effects of urbanization, forest size and edge effects slightly influenced total species richness and abundance but appeared to have a major effect on ground beetle assemblages through species specific responses. More urbanized sites had significantly fewer forest specialists and more generalist species. Large forest fragments were favored by forest specialist species while generalist species and species frequently associated with forest (forest generalists) dominated the smaller forests. Forest edges mainly harbored generalist species while forest specialist species were more frequent into the forests if the forest patches were large enough, otherwise they disappeared due to the destruction or impoverishment of their habitat. Our results show the importance of differentiating between habitat affinity, especially habitat generalists versus specialists, the latter having a higher value in nature conservation, and merely the quantity of species represented in human-dominated areas.

Keywords: landscape ecology, urbanization, edge effects, species diversity, fragmentation, habitat affinity

2.2 Introduction

Forest fragmentation is an important process contributing to the present-day concern for the loss of biodiversity and increased rates of species extinction (Didham et al., 1996). The most important and largest-scale cause of changes in the degree of fragmentation is anthropogenic habitat modification (Fahrig 2003), and this is especially pronounced in urban areas, where increased ‘sprawl’ is a significant environmental issue for the 21st century (Dwyer et al. 2003, Hunter 2003). Urban expansion is a problem that continues to impact ecological interactions among species (Salleh & Galante 2003). Replacement of natural habitats by human-dominated areas has resulted in habitat loss and isolation of remnant habitat patches (Rickman & Connor 2003, Primack 2006). If we want to understand how biodiversity is affected by urbanization, it is important to study the response of organisms to human-caused disturbance (Grandchamp et al. 2000), especially anthropogenic habitat fragmentation.

Fragmentation *per se* is a landscape level phenomenon, which not only reduces habitat size and quality but increases isolation and creates ecological boundaries that differ significantly from true core habitat. The implications of this for individual organisms are many and varied, because species with differing life history strategies are affected differently (Ewers & Didham 2006). Besides the effects of habitat loss, organisms that remain in fragments experience conditions more similar to those in the surrounding habitats. These so-called ‘edge effects’ have a significant impact on species living in the remaining habitat patches (Rolstad 1991, Saunders et al. 1991, Murcia 1995). To counteract the negative effects of the surrounding matrix, it is necessary to know how species respond to conditions in habitat edges (Haila et al. 1994). The environmental conditions in the surrounding matrix also affect the survival potential of species that are reliant on the original forest habitat. The surrounding matrix can be a source of opportunist species that may invade (Tilman et al. 1994, Tscharntke et al. 2002) habitat remnants and consequently modify species composition and community structure (Schoereder et al. 2004). Hence, preserving sufficient interior habitat is not the only important factor to protect these forest interior species and maintain biodiversity (Burke & Goulet 1998, Murcia 1995), as the environment between remnants might also alter the forest species composition. Moreover, recent empirical work demonstrated that a synergistic interaction between habitat area and distance to the edge was a more important determinant of patterns in beetle community composition than direct edge or area effects per se (Ewers et al. 2007), although the influence of differing surrounding matrices in an urban context has been less well studied (Watson et al. 2005).

The present study is based on the concept of populations inhabiting suitable habitat patches surrounded by less suitable or non-suitable habitat and is part of the international Globenet program (Niemelä et al. 2000), which was created to investigate the effects of urbanization on ground beetles in forest fragments. Carabid beetles were selected as a reliable monitoring group because they are ecologically and taxonomically sufficiently varied, abundant and sensitive to human-caused disturbances (Desender et al. 1991, Desender et al. 1994, Desender 1996, Luff 1996, Niemelä 1996, Dufrêne & Legendre 1997, Niemelä et al. 2000). In addition to being indicators, carabid beetles themselves may be targets for conservation efforts (Niemelä 2001) because many species are threatened (Desender & Turin 1989). The aim of the work was to understand how woodland size, edge characteristics and the degree of urbanization in the surrounding habitat matrix, affects carabid beetles. We considered the responses of carabid beetles at three levels: abundance and species richness of the total community, abundance and species richness of the different habitat affinity groups and abundance at species level. We address the following questions: (i) How does the degree of urbanization in the surrounding habitat affect woodland beetles and does it interact with the patch size and distance to the edge? (ii) Could responses of individual species or habitat affinity groups be masked by patterns observed at higher assemblage levels?

2.3 Materials and Methods

Study area

Flanders, the northern part of Belgium, as well as other regions in Western Europe, is scarcely covered with forests (8%). This region has a long history of intense agricultural exploitation and high population densities, which resulted in a complex cultural landscape with a dense urban and infrastructural network. The Brussels Capital in Belgium has a surface area of 160 km^2 and a very high population density of around one million inhabitants. Further expansion of the region is a considerable threat to the remaining natural habitats (Gryseels, 1998).

Our investigations were carried out in 10 forests situated in Brussels (50°74’-50°89’N, 4°29’-4°41’E), Belgium (Fig 1). The sites selected for study have: (i) a similar soil type (loam), (ii) beech (*Fagus*) dominated stands, (iii) >230 years old (i.e. since the maps of de Ferraris, 1775), (iv) no evidence of recent severe anthropogenic disturbances and (v) a similar southwest orientation of the edges because edge orientation might potentially affect the magnitude of edge effects (Fraver, 1994; Murcia, 1995; Honnay et al., 2002).

Sampling was performed in three different areas in the city. The first set of sites were situated in the Soignes forest (4383 ha). This is the largest area of forest in the northern part of Belgium accounting for 60% of the Brussels public green areas. The second set were in the southeast of Brussels, the relics of Soignes, also with old trees that, prior to its definitive protection (since 1842), were part of large private estates and were later incorporated into prosperous neighborhoods of villas or large forested parks. The third set of sites were in the northwest (Jette) of the city, and originates from afforestation in the 18th century of old calcareous limestone quarries. In total we investigated 13 sites that differed in degree of urbanization (Fig 1). The percentage of urbanization (Table 1) was objectively calculated for each site and as such used in the analyses (see urbanization measurements).

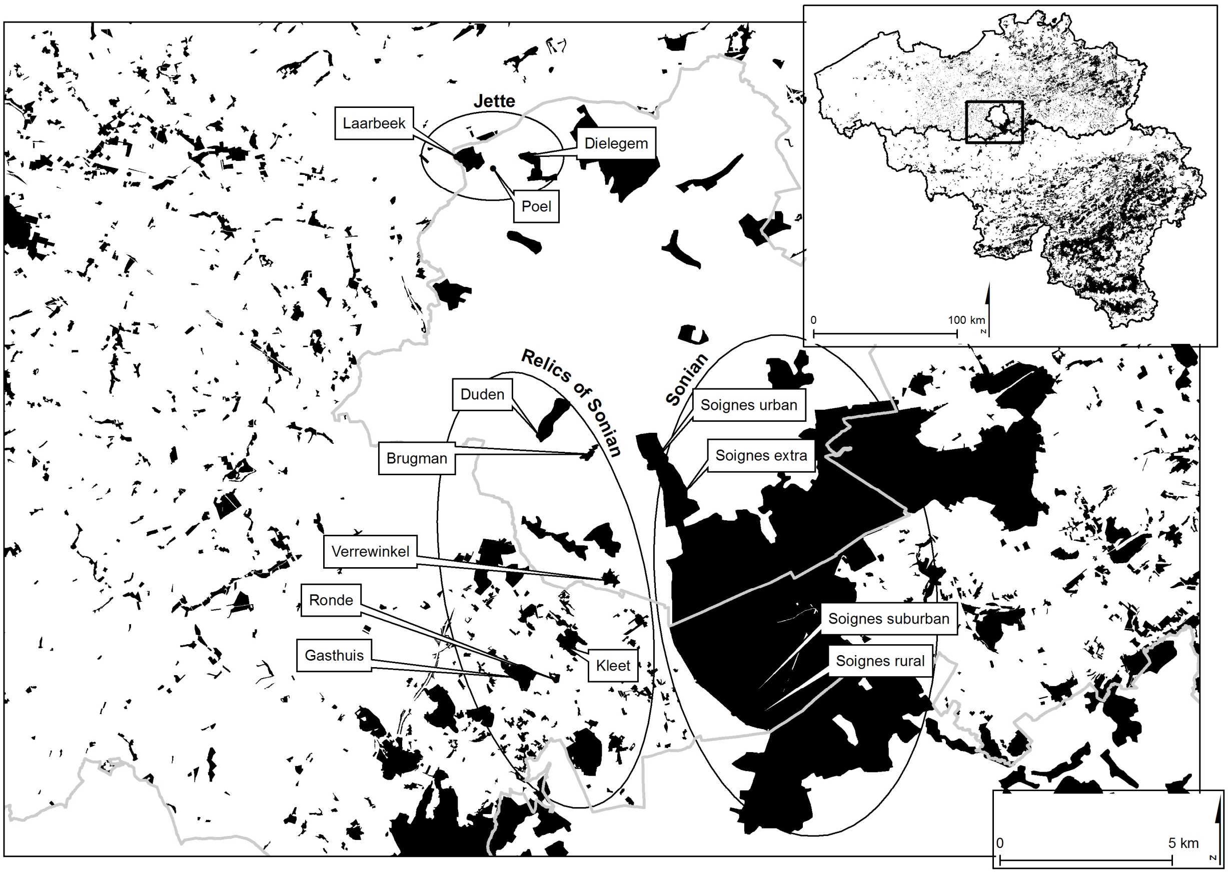

Fig. 1 – Sampling locations in and around Brussels, the capital of Belgium (in right upper frame). The dark areas are forest.

Sampling

Carabid beetles were collected using pitfall traps. Pitfall traps (mouth diameter = 95mm, 90mm deep) were arranged in line transects at each site, with traps running perpendicular from 30 m outside the forest (if possible), at the forest border (0 m), and at 30 m, 60 m and 100 meters into the forest. At each distance from the forest edge a trap line of three pitfalls, parallel to the forest edge, and 5 m apart, were installed. In total, 174 pitfalls were installed (Table 1) filled with a 4% formaldehyde solution to kill and preserve the collected arthropods. Forests were sampled from 1 March 2002 until 19 November 2002 and traps were emptied fortnightly.

Table 1 – Site description of the 13 sampling locations (plot), studied throughout the 2002 growing season

Plot	Area(ha)	Urbanization	Edge gradient	Pitfalls(nr)	History
Brugman park	5.3	90.32 %	-30/0/30/60	12	Relic of Soignes forest
Duden park	21.3	75.62 %	0/30/60/100	12	Relic of Soignes forest
Dielegem park	15.2	74.99 %	0/30/60/100	12	Former lime clearing
Soignes urban	88.2	59.31 %	0/30/60	9	Soignes forest
Poel forest	7.5	56.76 %	-30/0/30/60/100	15	Former lime clearing
Ronde forest	5.4	56.09 %	-30/0/30/60	12	Relic of Soignes forest
Verrewinkel forest	13.9	43.99 %	-30/0/30/60/100	15	Relic of Soignes forest
Kleetforest	43.1	42.11 %	-30/0/30/60/100	15	Relic of Soignes forest
Soignes extra	88.2	35.03 %	-30/0/30/60	12	Soignes forest
Soignes suburban	4383.3	25.80 %	-30/0/30/60/100	15	Soignes forest
Gasthuis forest	38.9	22.32 %	-30/0/30/60/100	15	Relic of Soignes forest
Laarbeek forest	37.9	15.52 %	-30/0/30/60/100	15	Former lime clearing
Soignes rural	4383.3	13.45 %	-30/0/30/60/100	15	Soignes forest

Urbanization measurements

The degree of urbanization at each site was calculated by means of digitized maps, incorporated into ArcView GIS (Environmental Systems Research Institute, Copyright 1992-1999, Version 3.2). A buffer of 840 m, being virtually the largest possible circle containing only forest surface in Flanders[1] was constructed around each sample site. Vegetation, land-use and small landscape elements were mapped for the entire Flemish and Brussels region using a uniform legend comprising some 120 units in the digital Biological Valuation Map. As an integrated measure of urbanization, we grouped the anthropological units: buildings, pavement, roads and railways which are intolerable habitat to ground beetles and determined the percentage of surface covered by these units within each buffer.

Statistical analyses

In a first set of analyses, we investigated whether species richness and total abundance were related to patch size, urbanization and forest edge by means of generalized linear mixed models (GLMM) using SAS 9.1.3. Catches from three pitfalls (line transect) were pooled over the entire trapping period. The response variables were abundance and species richness and the predictor variables were forest size, percentage urbanization and distance to the forest edge. Species or catch responses appeared to vary nonlinear with distance to forest edge, and we therefore included the quadratic term distance*distance. Patch size was log transformed in order to reduce the leverage of very large forests (e.g. Soignes) compared to the other forests in the regression analysis. To correct for possible differences between regions (Jette, Relics of Soignes and Soignes) and between forests, region and forest were included in the model as random factors in which forest was nested within region. However, the random effects, region and forest, did not explain a significant part of the variation and were excluded from the analysis. Subsequently, generalized linear models (GLM) were used for all analyses. The interaction forest size*distance from the edge was not significant in any analysis and was dropped from the analyses. Abundance data were modeled as a (overdispersed) Poisson distribution and species number was modeled following a normal error distribution.

In a second set of analyses, we investigated whether species composition differed as a function of forest patch size, urbanization and distance to the forest edge. Ground beetle species were pooled according to their habitat preference into four groups (cf. Sadler et al. 2006). A distinction was made between forest specialist species (limited to specific forest types), forest generalist species (occurring in many forest types including ancient as well as recent forest), open landscape species, and generalist species (occurring in both open landscape and forest) using previous data from all major habitat types in Belgium (Desender et al. 1995, Turin 2000). Finally, the response of the three most abundant beetle species per habitat affinity group was analyzed in detail to explore species specific response patterns. The errors of the individual species and habitat affinity groups were modeled following an overdispersed Poisson distribution. For open landscape species, only one species (*Trechus*

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[1] De Vos B., personnal communication

quadristriatus), entering regularly into the forest, was analyzed. Other open landscape species entered the forest only occasionally and therefore forest fragment size and distance to the forest edge were not meaningful parameters in these cases and were withdrawn from further analyses.

2.4 Results

We collected 53594 individuals across the urban-rural gradient in Brussels, corresponding to 100 carabid species (Table in Appendix). A total of 11 species (827 individuals) were forest specialists, 17 species (21240 individuals) forest generalists, 37 species (29509 individuals) generalist species, while 35 species (2018 individuals) were open-habitat species. Overall, the most abundant species were *Nebria brevicollis* and *Pterostichus madidus* (46.62% and 21.57% of the total catch respectively).

Species richness and total abundance

Species richness decreased with increasing levels of urbanization, irrespective of the distance of the traps from the forest edge Fig 2A and Table 2). The effect of forest size on species richness was less straightforward as it depended on urbanization. The effect of forest size appeared to be only present in less urbanized sites, where large forests harbored more species compared to smaller forests (Fig 2B and Table 2).

In contrast to the positive effect of forest size in rural sites on species richness, the abundance was lower in large forests (Fig 2A and Table 2).

Conclusively, urbanization had a negative effect on both abundance and species richness. Forest size only had an effect in the less urbanized, and hence species rich sites, wherein large forests were characterized by a community with lower abundance, but more species richness.

Abundance of habitat affinity groups

After species were pooled according to their habitat affinity group, the abundances of all four groups decreased significantly with increasing urbanization pressure (Fig 3A and Table 2). Increasing forest size appeared to have a positive effect only for forest specialists, while the number of open landscape and even forest generalist individuals decreased with increasing forest fragment size (Fig 3A and Table 2). For generalists, the effect was only present in rural sites, as indicated by the interaction between forest size and urbanization (Fig 3A and Table 2).

As expected, forest specialist abundance increased significantly with increasing distance from the forest edge while open landscape beetles significantly decreased (Fig 3B and Table 2).

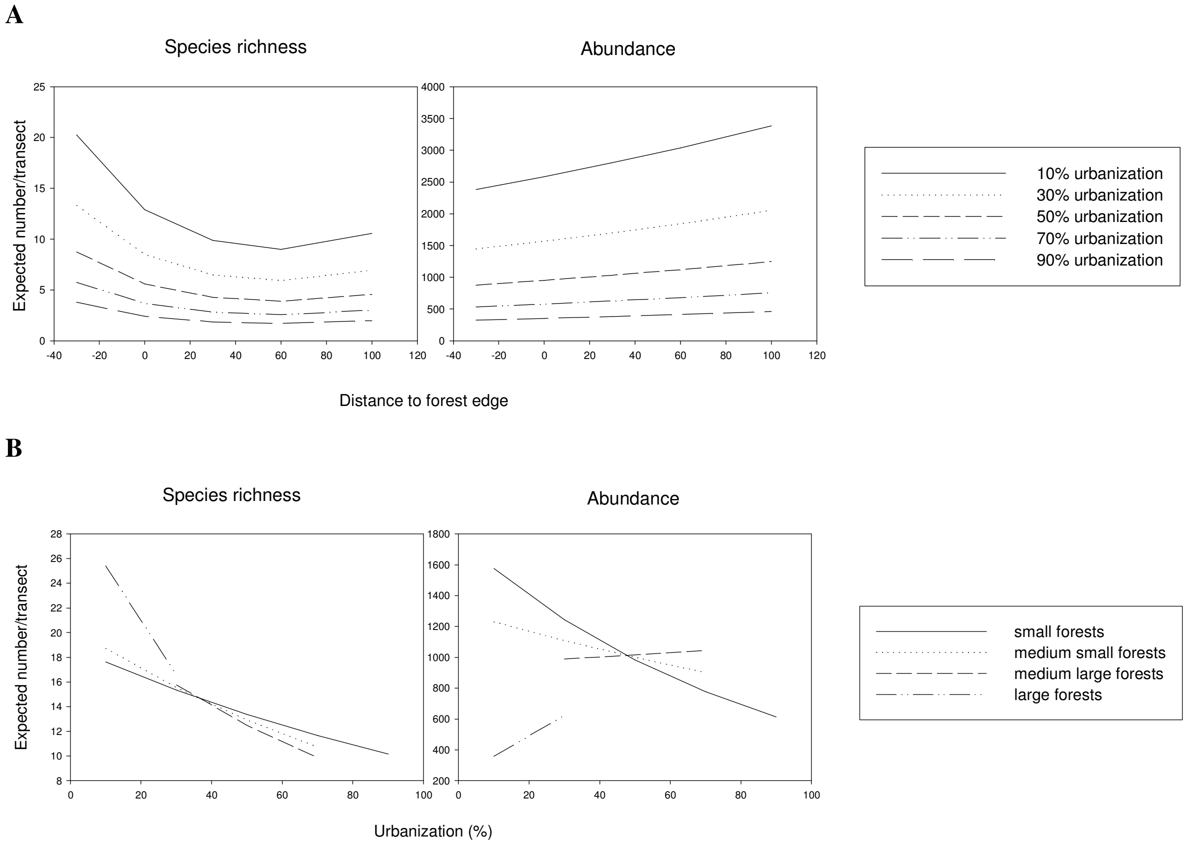

Fig. 2 – (A) Modeled expected number of species (left) and total abundance (right) per pitfall trap in function of the distance to the forest edge with differing urbanization degrees. 10 % urbanization in solid lines, 30 % in dotted lines, 50 % urbanization in short dashed lines, 70 % urbanization in dashed-dotted lines and 90 % urbanization is long dashed lines. (B) Modeled expected number of species (left) and total abundance (right) per pitfall trap along the urbanization gradient with differing forest sizes. Small forests (<20 ha) in solid lines, medium small forests (between 20-50 ha) in dotted lines, medium large forests (between 50-150 ha) in dashed lines and large forests (>150 ha) in dashed-dotted lines.

Analyses at species level

The effect of urbanization was very similar for all analyzed model species irrespective of their habitat affinity type. These numbers decreased with increasing degree of urbanization (Fig 4 and Table 2). Only for *Carabus auronitens* and *Pterostichus madidus*, this effect was not significant (Table 2).

Two of the three forest specialist model species (*Carabus auronitens* and *Cychrus attenuatus*) showed the same response to urbanization and forest size as the total abundance of this habitat affinity group. They were only present in large forests, which were also characterized by a low degree of urbanization. For *Pterostichus cristatus*, there appeared to be a preference for large forests only when the degree of urbanization is high (positive urbanization*forest size effect). This result may however be confounded with its presence only in Soignes forest sites (Appendix 2).

For the forest generalist species, *P. madidus* and *Carabus violaceus,* we observed lower abundances in larger forests. Moreover, for *C. violaceus*, the effect of urbanization was reversed in small forests compared to large forests.

The generalist model species responded more or less similar to the forest generalist group, but the effect of forest size was much more pronounced. Smaller forests contained much more individuals of the three model species especially when the degree of urbanization was low.

As expected, the abundance of forest generalist (*Pterostichus madidus*, *Abax ater* and *Carabus violaceus*) as well as forest specialist (*Carabus auronitens*, *Pterostichus cristatus* and *Cychrus attenuatus*) model species increased with increasing distance to the forest edge, which is in accordance to their habitat affinity type (Fig 5 and Table 2). Concerning generalists, the pattern described for *Nebria brevicollis* is almost identical to the pattern observed in the generalist group. This could be expected as this species is by far the most abundant generalist in our study and therefore completely determines the response of the generalist group. *Bembidion lampros* and *Pterostichus strenuus* showed a highly similar response that was different from *N. brevicollis*. These were most numerous in small forests with low degree of urbanization. (Fig 5 and Table 2)

Table 2 – The effects of urbanization, forest patch size and distance to the forest edge on species richness, abundance, habitat affinity groups and individual species as analyses by means of GLMM with log link

The given values are the coefficients with their standard errors, both on a log scale, followed by the significance level: *** p=<0.001, ** p=<0.01, * p=<0.05). The three factors were continuous variables mentioned in table 1, urbanization (in percentage), distance to the edge (edge gradient) and size (area (ha) on a logarithmic scale in the analyses).

	Urbanization	edge distance	edge distance^2 (quadratic term)	forest size	urbanization*forest size	urbanization*distance
SPECIES	-0,0021± 0,0036	-0,012 ± 0,0015***	0,0001 ± 0,0000***	0,085 ± 0,0301**	-0,0024 ± 0,0011*	
ABUNDANCE	-0,025 ± 0,0098**	0,0027 ± 0,0023		-0,3134 ± 0,109***	0,0066 ± 0,0030*	
FOREST SPECIALIST	-0,0382 ± 0,0119***	0,0074 ± 0,0027**		0,4806 ± 0,0661***		
Carabus auronitens	-0,0224 ± 0,0149	0,0338 ± 0,0110**	-0,0002 ± 0,0001*	0,4511 ± 0,0924***		
Pterostichus cristatus	-0,0854 ± 0,0337*	0,0554 ± 0,0153***	-0,0003 ± 0,0001**	-0,1082 ± 0,1910	0,0277 ± 0,0068***	-0,001 ± 0,0004*
Cychrus attenuatus	-0,1433 ± 0,0235***	0,0318 ± 0,0076***	-0,0002 ± 0,0001**	0,5631 ± 0,0955***		
FOREST GENERALIST	-0,0132 ± 0,0047**	0,0035 ± 0,0024		-0,1649 ± 0,0552**		
Pterostichus madidus	-0,0053 ± 0,0060	0,0307 ± 0,0079***	-0,0002 ± 0,0001**	-0,2292 ± 0,0839**		
Abax ater	-0,0139 ± 0,0068*	0,0303 ± 0,0102**	-0,0002 ± 0,0001*	-0,0394 ± 0,0803		
Carabus violaceus	-0,0739 ±0,0218***	0,0378 ± 0,0121**	-0,0002 ± 0,0001*	-0,5539 ± 0,1601***	0,0195 ± 0,0057***	-0,0005 ± 0,0002*
GENERALIST	-0,0358 ± 0,0138**	0,0019 ± 0,0030		-0,5274 ± 0,1655***	0,0123 ± 0,0041**	
Bembidion lampros	-0,044 ± 0,0086***	-0,0614 ± 0,0075***	0,0004 ± 0,0001*	-0,1549 ± 0,0634**		
Nebria brevicollis	-0,0362 ± 0,0127**	0,0178 ± 0,0060**	-0,0002 ± 0,0001*	-0,5216 ± 0,1514***	0,013 ± 0,0038***	
Pterostichus strenuous	-0,0584 ± 0,0130***	-0,0229 ± 0,0082**	0,0003 ± 0,0001**	-0,4636 ± 0,1471**		
OPEN LANDSCAPE	-0,0768 ± 0,0122***	-0,014 ± 0,0049**		-0,3411 ± 0,0985***		
Trechus quadristriatus	-0,0827 ± 0,0136***	-0,0664 ± 0,0107***		-0,1251 ± 0,0636*		

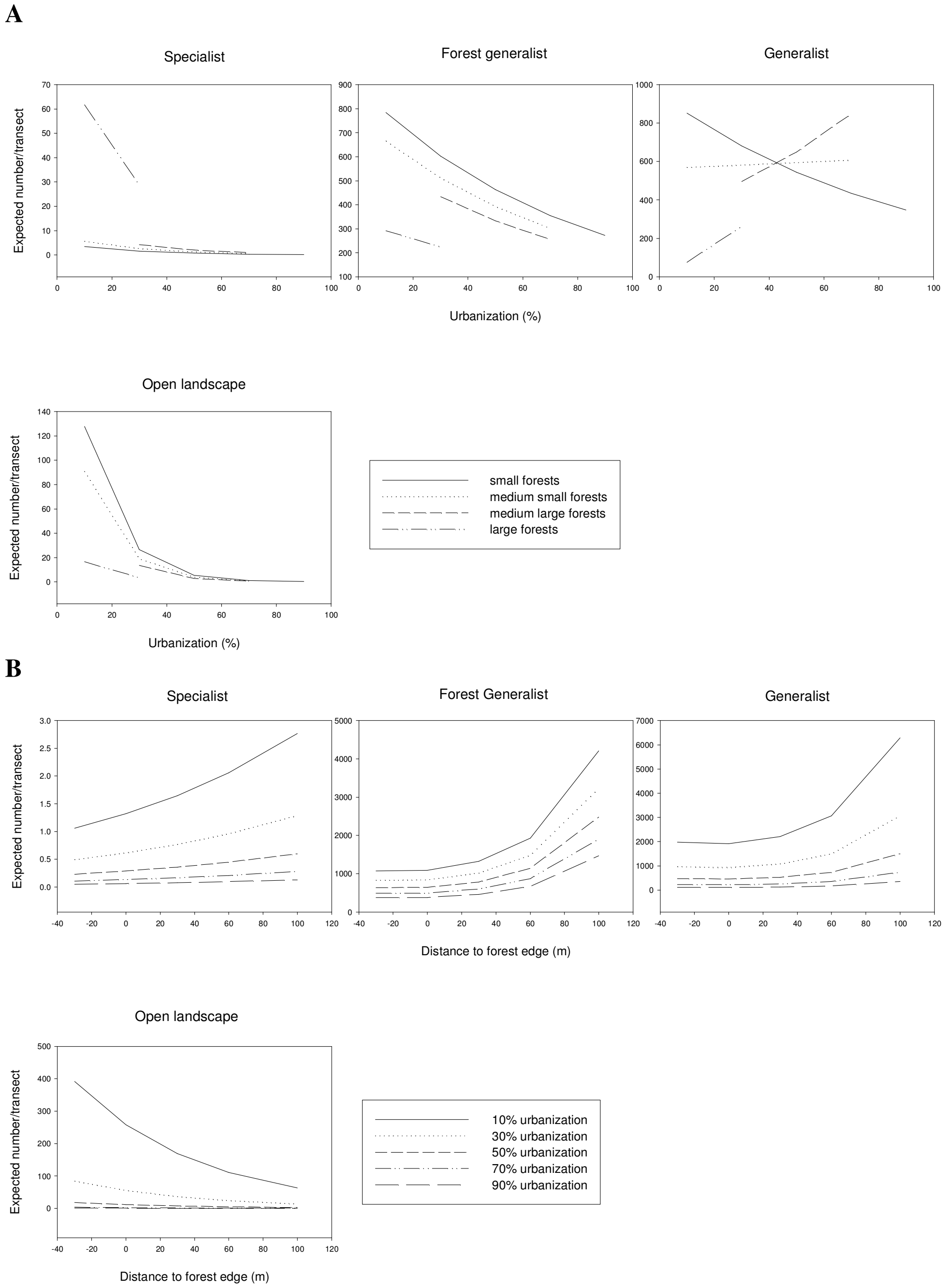

Fig. 3 – (A) Modeled expected number of individuals per pitfall trap of the four different habitat affinity groups (specialist, forest generalist, generalist and open landscape) along the urbanization gradient in function of the distance to the forest edge with differing urbanization degrees. 10 % urbanization in solid lines, 30 % in dotted lines, 50 % urbanization in short dashed lines, 70 % urbanization in dashed-dotted lines and 90 % urbanization is long dashed lines with differing forest sizes. (B) Modeled expected number of individuals per pitfall trap of the four different habitat affinity groups (specialist, forest generalist, generalist and open landscape) along the urbanization gradient with differing forest sizes. Small forests (<20 ha) in solid lines, medium small forests (between 20-50 ha) in dotted lines, medium large forests (between 50-150 ha) in dashed lines and large forests (>150 ha) in dashed-dotted lines.

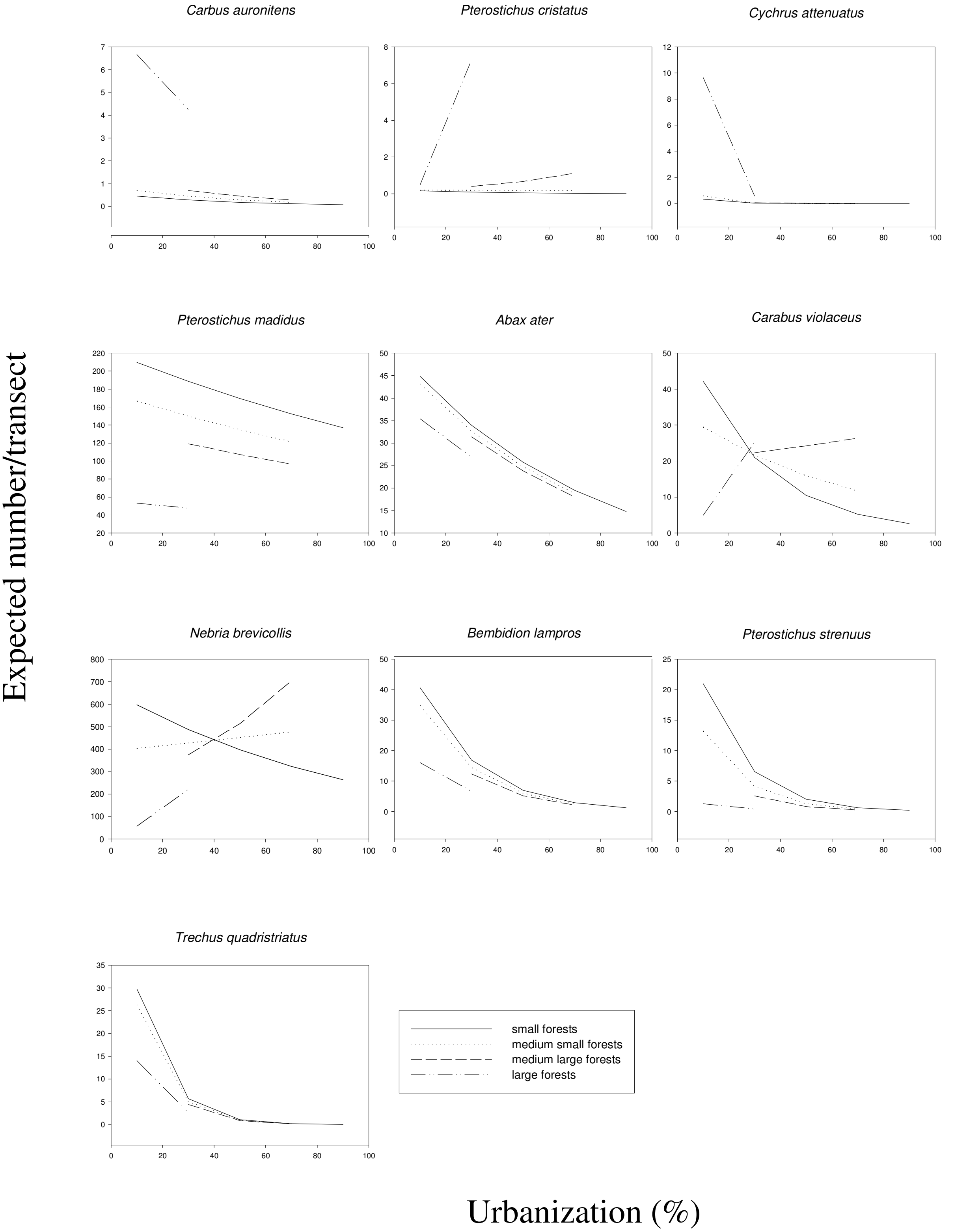

Fig. 4 - Modeled expected number of individuals per pitfall trap of selected species along the urbanization gradient with differing forest sizes. Small forests (<20 ha) in solid lines, medium small forests (between 20-50 ha) in dotted lines, medium large forests (between 50-150 ha) in dashed lines and large forests (>150 ha) in dashed-dotted lines.

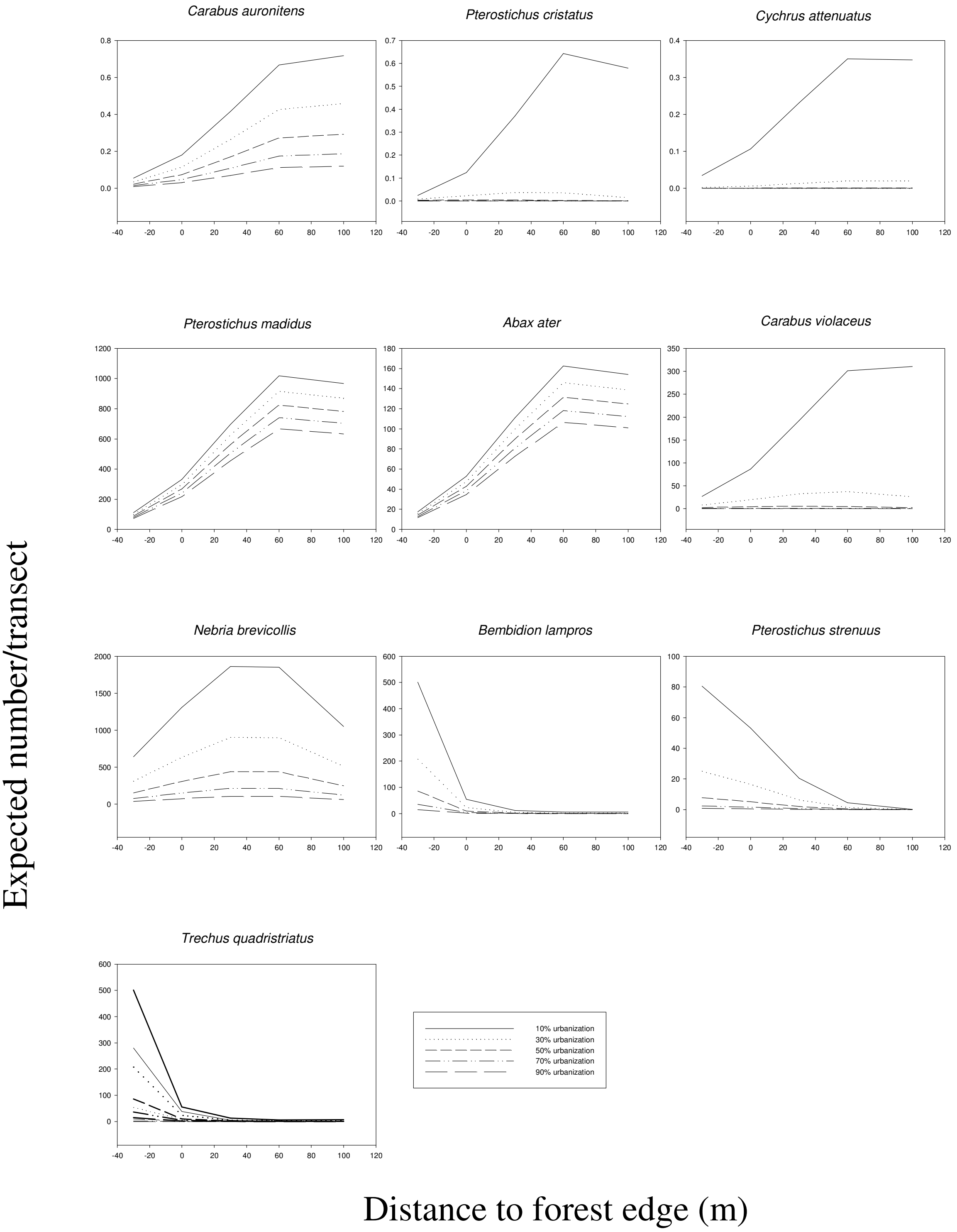

Fig. 5 - Modeled expected number of individuals per pitfall trap of selected species along the urbanization gradient in function of the distance to the forest edge with differing urbanization degrees. 10 % urbanization in solid lines, 30 % in dotted lines, 50 % urbanization in short dashed lines, 70 % urbanization in dashed-dotted lines and 90 % urbanization is long dashed lines with differing forest sizes.

2.5 Discussion

The urban matrix, forest patch size and forest edge effect are three different effects of habitat fragmentation, which have rarely been analyzed together within the scope of one study. Our analyses were first performed on total abundance and species richness but did not show consistent results. More detailed analyses account for the habitat affinity of the species and single species, and led to much more convincing and clearer conclusions. Urbanization has a significant effect on carabid beetles, but these effects are complicated by edge effects and patch size. Moreover, the results differ according to the level at which the community is analyzed (i.e. whole assemblage, habitat affinity groups and species level).

The influence of the matrix on ground beetles

Forest fragments are surrounded by a matrix which is different from the forest patch itself. We have demonstrated that the amount of urbanization within the matrix has a significant effect on the carabid beetle abundance, as well as on assemblages populating these forest fragments. Arthropods show varied responses to urbanization, meaning that some arthropod communities show increases in urban habitat fragments while other arthropod communities decline (Kitahara & Fuji 1994, Bolger et al. 2000, Alaruikka et al. 2002). Species richness is often high in urban habitats but for some groups of organisms, urban conditions are not favorable (Niemelä 1999). Species richness of ground dwelling carabids is not favored by urbanization. Carabid abundance, in our study, also declined with increasing urban pressure. In recent studies carabid abundance and species richness also decreased with increasing urbanization in Edmonton (Canada), Helsinki (Finland) (Niemelä et al. 2002, Venn et al. 2003), Hiroshima (Japan) (Ishitani et al. 2003) and Birmingham (UK) (Sadler et al. 2006).
However, simply counting the number of species provides no information on species assemblages and species value and may even be misleading because disturbances may favor widespread species, leading to an increase in richness (Margules et al. 1994, Davies & Margules 1998). Taking into account the habitat preferences of different species showed that urbanization has detrimental effects on the abundances of all habitat affinity groups, which indicated a higher influx of individuals from surrounding habitats in the rural environment. Our sampling design allows us to demonstrate that generalists and open landscape species are indeed more numerous outside the forest in rural environments (Appendix 2). All sample sites were ancient forest, but increased urbanization of the surrounding environment, had an important negative influence on beetle abundance. Examination of insect communities in

urban areas suggested that the response of insects to urbanization is species specific (Alaruikka et al. 2002, Rickmann & Connor 2003). In our study, the abundance of the vast majority of studied species was negatively affected by urbanization, showing significant decreases in more urbanized sites. *Bembidion properans* was an exception and was favored by urbanization. This could be due to the specific habitat preferences of this beetle to parks, gardens and lawns and its higher dispersal capacities through flight (Desender & Pollet 1986, Desender 2000). The degree of specialization of different species can hence be an important factor determining their susceptibility towards urbanization. In this study, more specialized species appeared to be more sensitive than generalist species, likely because they show little flexibility to cope with a changing environment (Didham et al. 1996, Magura et al. 2004, Sadler et al. 2006).

The influence of forest patch size and edge distance on ground beetles

Forest patch size showed a differentiated effect: large forest fragments were only favored by forest specialist species while smaller fragments were favored by generalist and forest generalist species. The positive effect of reduced patch size relates to generalist species that are not specifically bound to a particular habitat. Forest specialists (strongly attached to a certain habitat) occurred only in the larger fragments presumably due to their longer life spans, lower reproductive rates and limited dispersal which makes them very susceptible to local extinction (Turin 2000, Sadler 2006). Gibb & Hochuli (2002) suggested that differences in the composition of arthropod assemblages in small and large fragments also imply that species richness alone is too simplistic a measure of diversity when evaluating the complex changes occurring after fragmentation.

These results are linked to forest edge effects, here defined as responses in relation to the distance to the forest-adjacent habitat border. We were able to demonstrate an increasing number of forest specialist species with an increasing distance from the edge and a decreasing number of open landscape species deeper into the forest. The abundance of generalist and forest generalist species increased with an increasing distance from the forest edge. In the latter two groups, the abundance of all separately analyzed model species increased with an increasing distance to the forest edge, except for *Nebria brevicollis* which abundance decreased deeper into the forest. Therefore, the total response of the generalist species group was concealed by the most numerous species, *N. brevicollis,* and turned out not to be significant. Analysis at the species level showed highly significant but contradicting and species specific effects. This further documents the importance of analyzing at the species level instead of generalizing beyond species level. This was also demonstrated by Taboada et al. (2004) and Davies & Margules (1998) who observed no edge effect at the assemblage level while some individual carabid species were susceptible to edge effects and responded differently to the edge according to their differing habitat requirements.

In some studies, forest edges are supposed to function as source habitats (Pulliam 1988) or stepping stones (Den Boer 1990) for small scale dispersal processes after disturbances (Magura 2002) as edges contain species from both adjacent habitats (Kotze & Samways 2001). However, we noticed that edges can only harbor generalist species while forest specialist species with stringent habitat requirements will disappear after destruction or impoverishment of their habitat. Yet, these vulnerable species are, or should be, the most important target group for forest conservation management. Edge habitat has been recognized to be incompatible with the requirements of many forest species (Matlack & Litvaitis 1999). Forest fragments or corridors narrower than 100m across will be of limited value for the most sensitive specialist forest arthropods. Ozanne et al. (1997) suggested that edge penetration distance varies with taxon and that for some invertebrates effects are ameliorated rapidly within 3-6m of the edge, whereas for other taxa an effect can still be observed 25m into the stand. Management strategies employed for vertebrate conservation in fragmented habitats are unlikely to always suit invertebrates (Murphy & Wilcox 1986). The response of small organisms, which occur in a lower proportion at the edge, suggests that the dense forest structure provides more favorable conditions for many specialized forest invertebrates than the exposed edge. Small organisms are more likely to be sensitive to microclimatic stress such as low humidity and high wind speeds at the edge (Ozanne et al. 1997). These results suggest that habitat fragmentation may not lead to a loss of diversity in the short term, but rather to changes in community structure. It is possible that the declines recorded will eventually become local extinctions and that changes in species composition will result in further extinctions through biotic interactions, as the species that become more abundant have more influence on community dynamics.

Interactive effects between urbanization, patch size and edge effects

For species richness, the effects of urbanization and forest size were not independent. In rural sites species richness was higher in larger forests whereas in more urbanized sites there were more species in smaller forest stands. A small urban forest may be embedded in a varied matrix, proportionally having more edge, and surrounded by parks, gardens and lawns. Urban environments are more heterogeneous (Niemelä 1999) and therefore more species rich with especially opportunistic species, not strictly bound to forest. A rural small forest is mostly surrounded by one type of matrix, mostly pastures and/or crop fields. Therefore, in an urban environment, species coming from all these different surrounding matrices are more diverse, but less numerous than in a rural environment.

The interaction between urbanization and forest size also had a significant effect on total abundance, but in an opposite direction. Total abundance was lower in large forests when less urbanized but increased in large forests when more urbanized. This could be due to a higher productivity in more influenced urban forest, resulting in a higher number of individuals of generalist species. In an urban forest, species are generally smaller (Alaruikka et al. 2002, Ishitani et al. 2003) and smaller species can occur at higher abundances (Kotze et al. 2003).

In the generalist species group, the interaction between urbanization and forest size was also significant: more urbanized forest sites contained more individuals of generalist ground beetles if forests were large. This could be due to a lower heterogeneity in larger urban forests compared to small urban forests (Desender et al. 2005).

Forest size was more important in strongly (>50%) urbanized sites for the species *Nebria brevicollis*, *Carabus violaceus* and *Pterostichus cristatus*, with more individuals in larger forests. In an urban forest these species will possibly only occur if forests are large enough. This way they are protected against the deteriorating influences from the outer matrix in which they are not able to survive.

The interaction between urbanization and distance to the forest edge was significant for only two forest species (*Carabus violaceus* and *Pterostichus cristatus*), which were more numerous in the centre of less urbanized sites while distance did not play a major role in highly urbanized sites. These two forest species showed a clear preference for a habitat deeper inside the forest in a rural environment, while in an urban environment this appeared to be less important. Probably, the habitat in an urban environment is already completely deteriorated, from the edge to the interior, so the habitat is not suitable anymore for these species.

Interactions between forest size and distance to the forest edge were not significant in our study in contradiction to the study of Barbosa & Marquet (2002).

2.6 Conclusion

In the face of increasing human impacts, a major goal in conservation biology is to provide principles by which biological biodiversity can be preserved (Davies et al. 2000). However, with respect to maintaining biological diversity, it is important to differentiate between the value (rare and specialized versus common and generalist species) and the quantity (diversity) of the species that are represented in human-dominated areas (Blair 1996).

Forest specialist species are the most vulnerable group and should be considered the most important target group for forest conservation management. These specialist species with more specific habitat requirements are an appropriate focus of conservation (Rainio & Niemelä 2003).

Our findings confirm that more disturbed sites are significantly poorer in forest specialists. For these species, the maintenance and even restoration or creation of good quality habitat in the urban environment is the key to their continued survival rather than the more difficult task of increasing habitat connectivity (Angold et al. 2006). Forest specialist species may not be able to disperse via corridors or stepping stones. Habitat specialists also appeared to be more susceptible to extinction in smaller fragments than generalists. A forest patch needs to be of a minimum size to create conditions characteristic for forest interior, and this is not possible below a certain size (Lövei et al. 2006). Edges, relatively more present in small fragments, can only harbor generalist species

Negative effects of urbanization, small forest size and edge effects slightly influence species richness and abundance but have a major influence on species composition. Thus species turnover might be more important to be considered as compared to overall diversity values.

2.7 Acknowledgements

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2.9 Appendix

Appendix 2- Number of carabid beetles collected in each sample location ordered per site from most to least urbanized, based on the percentage of urbanization (left), as well as per distance from the edge (from 100 m into the forest to 30 m outside the forest (right). In the second column the habitat preference of each species is indicated (S=forest specialist, F=forest generalist, G=generalist and O=open landscape species.

	Habitat affinity	**Brugman park**	**Duden park**	**Dielegem park**	**Soignes urban**	**Poel forest**	**Ronde forest**	**Verrewinkel forest**	**Kleet forest**	**Soignes extra**	**Soignes suburban**	**Gasthuis forest**	**Laarbeek forest**	**Soignes rural**	**100**	**60**	**30**	**0**	**-30**
NUMBER OF TRAPS		12	12	12	9	15	12	15	15	12	15	15	15	15	27	39	39	39	30
Abax ater	F			4	388	497	149	199	226	407	61	417	64	450	602	857	866	490	47
Abax ovalis	S										206			210	161	95	134	14	12
Abax parallelus	S										1	3				1		3	
Acupalpus meridianus	G					4	1						1	1				1	6
Agonum albipes	O			1									76				75	1	1
Agonum assimile	F			163	3				8	11	14	11	958	2	104	91	894	53	28
Agonum dorsalis	O						24		1		1	41	72	40				7	172
Agonum muelleri	G			14	1	42	58	33	5	1	12	36	39	46	1	2	3	15	266
Agonum nigrum	O						3												3
Agonum obscurum	G					1							2				2		1
Agonum sexpunctatum	O													1					1
Amara aenea	G	11	1			18	10	3	4	5	3	2	7	20	1			8	75
Amara anthobia	O	1	2									1				1		2	1
Amara convexior	O								1								1		
Amara consularis	O		1															1	
Amara curta	O					1										1			
Amara eurynota	O						1											1	
Amara familiaris	G		1				1							1		1			2
Amar lunicollis	G						12		1		1	70					3	24	57
Amara ovata	O	1	1	1		2		3	1	6		2	1		3	1	3	1	10
Amara plebeja	G					3	3		3			22	10	18	2	1	4	16	36
Amara similata	O			1		2	2	3	1	1	1		1		2		1	1	8
Amara tibia	O							1											1
Anisodactylus binotatus	G					19	5	2	3		2	57	2	7			2	7	88
Asaphidion curtum	F	159	37	55	99	63	63	21	58	158	32	83	202	25	69	157	245	389	195
Asaphidion flavipes	G	4				61	4	8	2			9	39	44			5	3	163
Asaphidion pallipes	O													1					1

	Habitat affinity	Brugman park	Duden park	Dielegem park	Soignes urban	Poel forest	Ronde forest	Verrewinkel forest	Kleet forest	Soignes extra	Soignes suburban	Gasthuis forest	Laarbeek forest	Soignes rural	100	60	30	0	-30
Asaphidion stierlini	O					16		2	12			5	47	19		1		12	88
Badister bullatus	F	10	22	2	2	16	2	5	3	5	5	18	25	5	16	15	10	51	28
Badister lacertosus	G											1	3	1		1	1	1	2
Badister sodalis	G	1	4	4		7				2	2		8	2	9	7	6	7	1
Bembidion biguttatum	O			2			5						4			1		1	9
Bembidion deletum	S					3					2								5
Bembidion femoratum	O								1										1
Bembidion harpaloides	F												2			1	1		
Bembidion lampros	G	10	4	2	1	16	102	37	217	7	10	293	168	207	9	15	38	85	927
Bembidion lunulatum	G			1		1	24		9		1	7	87	28			1	2	155
Bembidion obtusum	O						90		11			13	181	42	2	4	16	20	295
Bembidion properans	O	6				15	327	1	12	2		41		81	1			3	481
Bembidion quadrimaculatum	O					10	5	12	19		1		21	74				5	137
Bembidion tetracolum	G	8	5	21		72	8		24	8	21	21	656	126	26	1	8	24	911
Bradycellus harpalinus	O		1					1				2			1				3
Bradycellus rufipes	O				1			2	2						3	1	1		
Bradycellus verbasci	G								1			9	1	1					12
Calathus fuscipes	G						1					1		44				1	45
Calathus melanocephalus	G													3					3
Calaths rotundicollis	F	20	71	12	162		9	8	45	4	5	58	106	10	81	227	107	94	1
Carabus auronitens	S									42	48			72	42	60	29	30	1
Carabus monilis	O													1				1	
Carabus nemoralis	F													5				3	2
Carabus problematicus	F										12	32		158	62	58	59	23	
Carabus violaceus	F				154		64	91	191	134	59	127	232	122	253	366	241	270	44
Clivina collaris	G	3				17	11	1	1			6		1				1	39
Clivina fossor	G						1		1	1		49	2	5					59
Cychrus attenuatus	S										11	5		74	27	27	19	17	
Demetrias atricapillus	G											2	3					1	4
Diachromus germanus	S						1						1						2
Dromius quadrimaculatus	F												1					1	
Dyschirius globosus	G								1									1	
Dyschirius intermedius	O					3								2					5
Dysc poli	O						1							1					2
Dyschirius thoracicus	O													1					1
Elaphrus cupreus	G												2				2		
Harpalus affinis	G	1		2		1	1				1	5		24	1			1	33

	Habitat affinity	Brugman park	Duden park	Dielegem park	Soignes urban	Poel forest	Ronde forest	Verrewinkel forest	Kleet forest	Soignes extra	Soignes suburban	Gasthuis forest	Laarbeek forest	Soignes rural	100	60	30	0	-30
Harpalus distinguendus	O					1								3					4
Harpalus latus	G						1	2	1			4		7	1	1	3		10
Harpalus rufipes	O						1		8			60	9	37	2	2	1	13	97
Leistus ferrugineus	G						2					2	2	1					7
Leistus fulvibarbis	F		2	5		5	1				5	6	32		6	4	22	16	8
Leistus rufomarginatus	F	11	27	15	23	2	14	4	59	9	10	46	28	19	72	92	69	33	1
Loricera pilicornis	G	3	1	28	5	4	25		62		14	42	119	18	13	12	83	33	180
Metabletus foveatus	O				1													1	
Molops piceus	S										1			11		1	9	2	
Nebria brevicollis	G	2176	2370	751	2894	610	2230	2325	4303	3390	432	1516	1708	283	4155	6944	7856	4526	1507
Nebria salina	O							1			1	5	1	17	5		1	7	12
Notiophilus biguttatus	F	81	46	30	40	9	42	18	268	76	34	128	134	42	142	270	312	180	44
Notiophillus palustris	G						6							2				1	7
Notiphillus quadripunctatus	S						2	1	9					1				6	7
Notiophillus rufipes	F	46	62	25	60	6	37	17	81	31	19	74	45	39	67	215	105	141	14
Notiophillus substriatus	O		1			8	17		2		1		7	15		1		6	44
Parophonus maculicornis	O					1	1				1	1							4
Patrobus atrorufus	S							1					43				43	1	
Pterostichus anthobia	O			1											1				
Pterostichus cristatus	S				2					14	35			12	13	15	21	13	1
Pterostichus cupreus	G			2			26	6	2		1	6	9	7	2	1	3	10	43
Pterostichus madidus	F	1151	29	1394	614	277	1153	1557	2350	743	171	1306	521	299	2454	3476	3546	1972	117
Pterostichus melanarius	G						1				5	34	319	112	6	13	14	88	350
Pterostichus minor	G			1														1	
Pterostichus niger	F								3		1		11	13	5	1	8	3	11
Pterostichus nigrita	G	1		2			4					2	37			3	36	4	3
Pterostichus oblongopunctatus	F				3				21		112	514	1	82	350	210	135	35	3
Pterostichus strenuus	G			15	8		10	1	5		6	45	64	1	38	8	29	18	62
Pterostichus vernalis	G	1					13	4	10		5	33	7	21	1			6	87
Pterostichus versicolor	G						25				1		1	5	1				31
Stenolophus teutonus	G					2	2					1		10			1	1	13
Synuchus nivalis	O						7	1	21			7			16	5	5	7	3
Trechoblemus micros	O						1					3							4
Trechus obtusus	G	3	10	18	2	3						1	23	2	24	10	2	11	15
Trechus quadristriatus	O							1	87	1		37	147	109	2	3	8	27	342
Trichotichnus laevicollis	S									1		15			15	1			
TOTAL		3708	2698	2572	4463	1818	4609	4372	8156	5059	1367	5337	6292	3143	8869	13281	15089	8854	7501

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Chapter 3

LOCAL EXTINCTION PROCESSES RATHER THAN EDGE EFFECTS AFFECT GROUND BEETLE ASSEMBLAGES IN FRAGMENTED OLD BEECH FORESTS.

Chapter 3

Local extinction processes rather than edge effects affect ground beetle assemblages in fragmented old beech forests.

EVA GAUBLOMME, HILDE EGGERMONT & FREDERIK HENDRICKX

3.1 Summary

The local extinction of specialist species due to fragmentation is one of the major causes of biodiversity loss. Such species are expected to suffer from both smaller local population sizes, which increase local extinction processes by increased effects of environmental or demographic stochasticity, and increased edge effects of fragmented patches. However, the relative effect sizes of these two factors are still poorly investigated. In this study, we attempt to disentangle these effects on carabid beetle communities of temperate broadleaved woodland fragments situated in one of the most urbanized regions in Belgium. Species assemblages were sampled by pitfall traps along transects that extended from 30 m outside to 100 m inside the fragment. Although species assemblages within the forest were highly distinct compared to those sampled outside the forest for all fragments, species turnover was less pronounced within forest fragments indicating only weak edge effects. The magnitude of edge effects did not differ significantly between large and small fragments. However, larger differences in species composition were observed with respect to fragment size, wherein highly specialized species persisted only in the largest fragment. In sum, local extinction processes, rather than edge effects, appeared by far more important to explain the absence of specialized forest species in this historic and fragmented woodland complex.

Keywords: insects, fragmentation, species turnover, matrix, community, area, carabidae, edge effects, extinction processes

3.2 Introduction

Different forms of anthropogenic land use result in an ever increasing fragmentation and isolation rate of the original habitat (Vitousek et al. 1997) and strongly affect species assemblages (Niemelä et al. 2002). Fragmentation is presumed to affect species composition of the focal patch by two different processes i.e. increased rates of local extinction by demographic and environmental stochasticity (Hanski 1998) and strong edge effects resulting from an increase in the proportion of edge to interior habitat (Fagan et al. 1999, Ries et al. 2004, Ewers et al. 2007). Invading species from the surrounding matrix, mainly good dispersing and generalist species, replace the forest specialist species, rendering small patches less suitable for the preservation of forest interior species (Ås 1999, Magura 2001, 2010, Summerville 2004 , Hendrickx et al. 2009, Lövei & Sunderland 2006). When edge effects penetrate deeply into the interior habitat, or when patches are very small, there may even be no interior habitat left (Laurance & Yensen 1991, Tscharntke et al. 2002, Ewers & Didham 2006). Given that both area *in se* and edge effects have a strong and interacting effect on ecological dynamics in fragmented landscapes (Tscharntke et al. 2002, Ewers et al. 2007), it is crucial to understand their relative role in determining species composition in order to effectively implement conservation strategies (Fletcher et al. 2007). Indeed, Fletcher et al. (2007) stated that most studies mix up the effects of fragment area and edge with only few studies effectively trying to separate them (e.g. Nour et al. 1993, Didham et al. 1998 a, b, Matthews et al. 1999, Barbosa & Marquet 2002, Galetti et al. 2003, Lienert & Fischer 2003, Fletcher 2005, Fletcher et al. 2007, Ewers et al. 2007, Banks-Leite et al. 2010).

Temperate broadleaved forests in Western Europe provide a good example of former relatively continuous habitat that suffered from dramatic levels of habitat fragmentation. In Belgium, forests have been under human pressure for about 7000 years. Their history is one of destruction, fragmentation and degradation, although there have also been some periods of forest recovery. Halfway the 19th century, woodland area reached its minimum (Tack et al. 1993).

Brussels - an industrialized region in Belgium - occupies 160 square km, holding a very high population density of around 1 million inhabitants. Despite the high urban character of the region, ancient forest fragments are still remnant (covering ~10% of the area), and harbour a valuable biodiversity (Gryseels 1998). Few of these fragments can be considered as large (i.e. >50 ha), but the majority is smaller than 50 ha, posing a serious threat to true forest specialist species (Hermy et al. 1999, Gaublomme et al. 2008). While most research in this region has been conducted on plants (Honnay et al. 1999, 2002), the distribution of arthropods within this region is less well-known and requires a more detailed study. This is crucial to enable realistic predictions how changes in their composition will be mediated by changes in the spatial configuration of the remnants due to future urban developments. In this study ground beetles were used as they form a well-known species-rich family that is widespread in all types of habitat (Lövei & Sunderland 1996). Studies on habitat edges, only focusing on species richness and abundance may be misleading, because disturbances may favor widespread and abundant species, leading to an increase in richness (Margules et al. 1994, Davies & Margules 1998, Gaublomme et al. 2008) and gives no information on the species composition.

In this study, we attempt to evaluate the effect of habitat edges and patch area on ground beetle assemblages by sampling a gradient, perpendicular to the forest edge, in both small and large fragments of a former contiguous forest area. More specifically, we address the following questions (i) To what extent determine edge habitat and forest size carabid assemblages of these forest fragments and (ii) Is the degree of species turnover from forest edge to forest interior related to forest size?

3.3 Materials and Methods

Study area

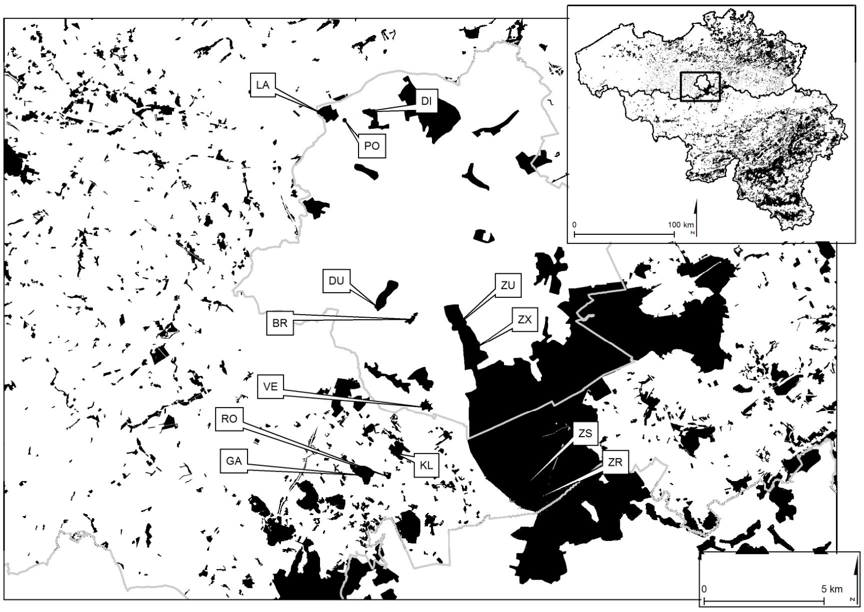

Fig. 1 - Sampling locations in and around Brussels (inset map shows the location of the sampling area in central Belgium). The dark areas represent forest habitat.

The study was conducted by comparing species composition of 13 transects distributed over ten different forest fragments, situated around the capital Brussels (50°74’-50°89’N, 4°29’-4°41’E) in Belgium (Fig 1, Table 1). In the large Soignes forest located in the southeast of Brussels, four different transects were studied whereas in the remaining forests, only one transect was sampled. To the west of the Soignes forest, we investigated six, relatively smaller remnant forests that were once connected to the Soignes forest. In the north of Brussels another three forests were selected, which were grown on former lime mining areas and have a different history compared to the other studied forests (Table 1). All the aforementioned forests (i) have a similar soil type (loam), (ii) are dominated by beech (*Fagus*), (iii) are more than 230 years old (i.e. since the maps of de Ferraris, 1775) and (iv) show no evidence of recent severe anthropogenic disturbances. Fragment size ranged from 5.3 ha to 4383 ha (Table 1). For all studied transects, the edge perimeter constitutes an abrupt boundary between forest vegetation and surrounding matrix, the latter ranging from arable field, meadow, park, garden to pavement. Fragment area was determined from digitalized maps in ArcView GIS 3.0 (Table 1).

Table 1 - Site description of the 13 forest fragments.

Forest fragment	Code	Area(ha)	Edge gradient	Pitfalls(nr)	History
Brugman park	BR	5.3	-30/0/30/60	12	Relic of Soignes forest
Duden park	DU	21.3	0/30/60/100	12	Relic of Soignes forest
Dielegem park	DI	15.2	0/30/60/100	12	Former lime mining
Soignes urban	ZU	88.2	0/30/60	9	Soignes forest
Poel forest	PO	7.5	-30/0/30/60/100	15	Former lime mining
Ronde forest	RO	5.4	-30/0/30/60	12	Relic of Soignes forest
Verrewinkel forest	VE	13.9	-30/0/30/60/100	15	Relic of Soignes forest
Kleetforest	KL	43.1	-30/0/30/60/100	15	Relic of Soignes forest
Soignes extra	ZX	88.2	-30/0/30/60	12	Soignes forest
Soignes suburban	ZS	4383.3	-30/0/30/60/100	15	Soignes forest
Gasthuis forest	GA	38.9	-30/0/30/60/100	15	Relic of Soignes forest
Laarbeek forest	LA	37.9	-30/0/30/60/100	15	Former lime mining
Soignes rural	ZR	4383.3	-30/0/30/60/100	15	Soignes forest

Beetle sampling and identification

Beetles were sampled using pitfall traps (mouth diameter = 95mm, 90mm deep) arranged in line transects at each site, running perpendicular from 30 m outside the forest (if possible), at the forest border (0 m), and at 30 m, 60 m and 100 m (if possible) into the forest. A distance of 100 m into the forest was the maximum achievable distance due to size restrictions in the smallest fragment. At each distance from the forest edge, a plot of three pitfalls were installed parallel to the forest edge, and the traps were placed with a 5 m distance from each other. All edges had a similar southwest orientation. A total of 174 pitfalls were set (Table 1), filled with a 4% formaldehyde solution to kill and preserve the collected arthropods. Traps were operational from 1 March 2002 until 19 November 2002 and emptied fortnightly. Beetles captured at each plot were pooled to obtain a single sample. All adults were identified to species level with the species identification key of Boeken et al. (2002).

Data analyses

Both unconstrained and constrained correspondence analysis (CA) were performed to investigate the relative importance of fragment area, distance along the transect, and the interaction between the two in determining beetle assemblage composition. After preliminary unconstrained ordinations, we performed some data manipulations as these resulted in a fairly homogeneous distribution of the plots. Given that rare species tend to have a large influence on the ordination results, we only included those species with total abundances larger than 58, corresponding to the number of plots. Species abundances were square root transformed to downweigh the effect of species whose abundances differ strongly among plots. Two species datasets were constructed, i.e. one including and one excluding all plots outside the forest habitat. The latter was constructed to obtain a more detailed picture of the differences within forest fragments as species composition of plots outside the forest differed remarkably from the interior assemblages (see results).

First, an unconstrained CA analysis was conducted to determine the most important variables that influence species gradients among all plots. Next, we constrained the CA analysis by the factors distance to forest edge (DIST), log transformed fragment area LN(AREA) and their interaction (DIST * LN (AREA)) and tested the significance of each by means of backward stepwise tests based on 10000 permutations. Ordination analyses and statistical tests were constructed with the vegan package (Oksanen et al. 2012) in R 2.15.0 (R Development Core Team 2009).

Since a constrained analysis on all plots only allows to test for consistent differences in species composition that are shared among all gradients with respect to distance, a second analysis was performed to estimate and test the rate of species turnover along the transects. Here, we calculated pairwise Jaccard dissimilarities between the plot located outside the forest, which is used as reference, and all interior plots for each gradient separately (Anderson et al. 2010). This resulted in maximum four independent measures of species turnover per gradient. Subsequently, we related these measures to DIST and the quadratic term DIST*DIST, log transformed area LN(AREA) and all respective interactions by means of a General Linear Mixed model with the mixed procedure in SAS v. 9.1.3. Transect ID was included as random effect, and fixed effects estimates of distance along the transects therefore return the rate of species turnover conditional on transects.

3.4 Results

A total of 53594 adult beetles were collected and yielded a total of 100 species, being about one quarter of all known ground beetle species in Belgium (Desender et al. 2008).

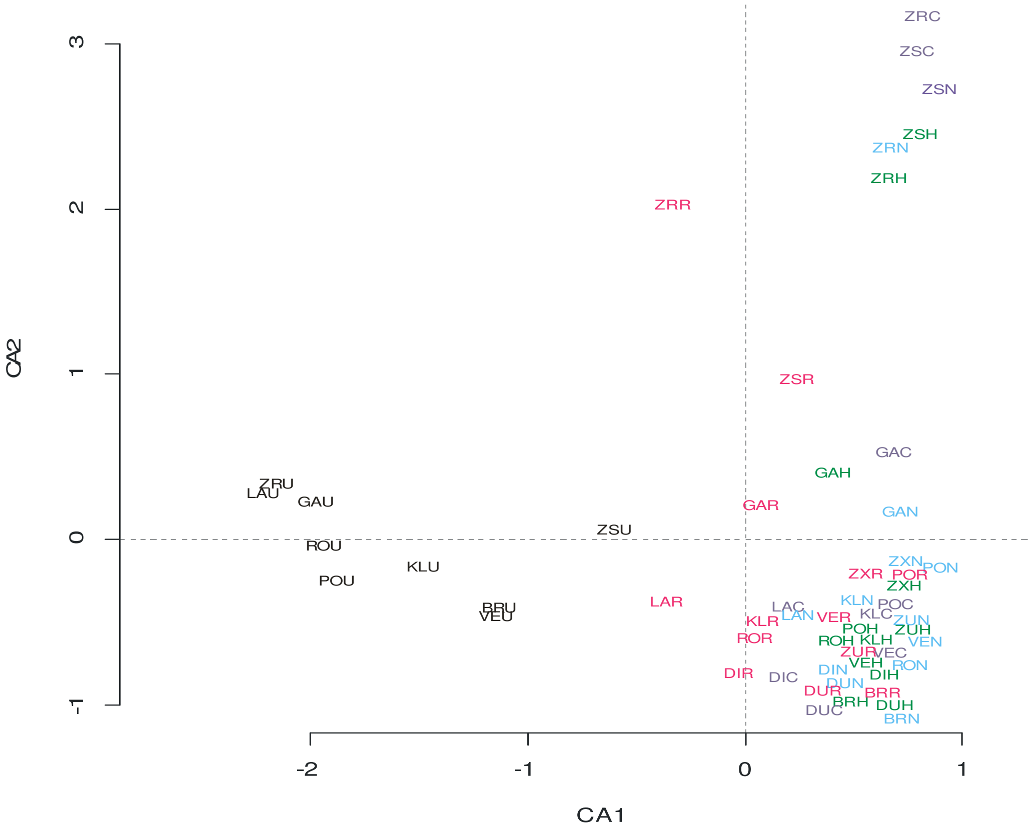

Fig. 2A - CA sample plot: eigenvalue axis 1 = 0.514, eigenvalue axis 2 =0.272. First two letters refer to the forest name (Table 1); last letter refers to the distance along the transect (U=-30 black label, R=0 red label, H=30 green label, N=60 blue label, C=100 purple label)

Multivariate analysis

An unconstrained CA analysis, based on all sample plots, indicates that the most important source of the total variation in species composition (total inertia = 1.575) depicted by variation along CA1 (eigenvalue = 0.514) is the separation of traps located outside the forest compared to those inside the forest habitat (Fig 2A). Samples located at the edge of the forest habitat tend to be situated much closer to those inside the forest habitat and showed that changes in species composition are rather steep along the transects (Fig 2A). Along the second axis (eigenvalue = 0.272), a clear and distinct separation is observed between the interior plots of the large continuous Soignes forest and the smaller forest fragments. The latter group also includes the fragments of Soignes that are only recently separated by urban infrastructure (ZX-ZU). Species that were found almost uniquely in all transects of the large continuous fragment are *Abax ovalis*, *Carabus problematicus*, *Carabus auronitens*, *Pterostichus cristatus* and *Cychrus attenuatus* (Fig 2B).

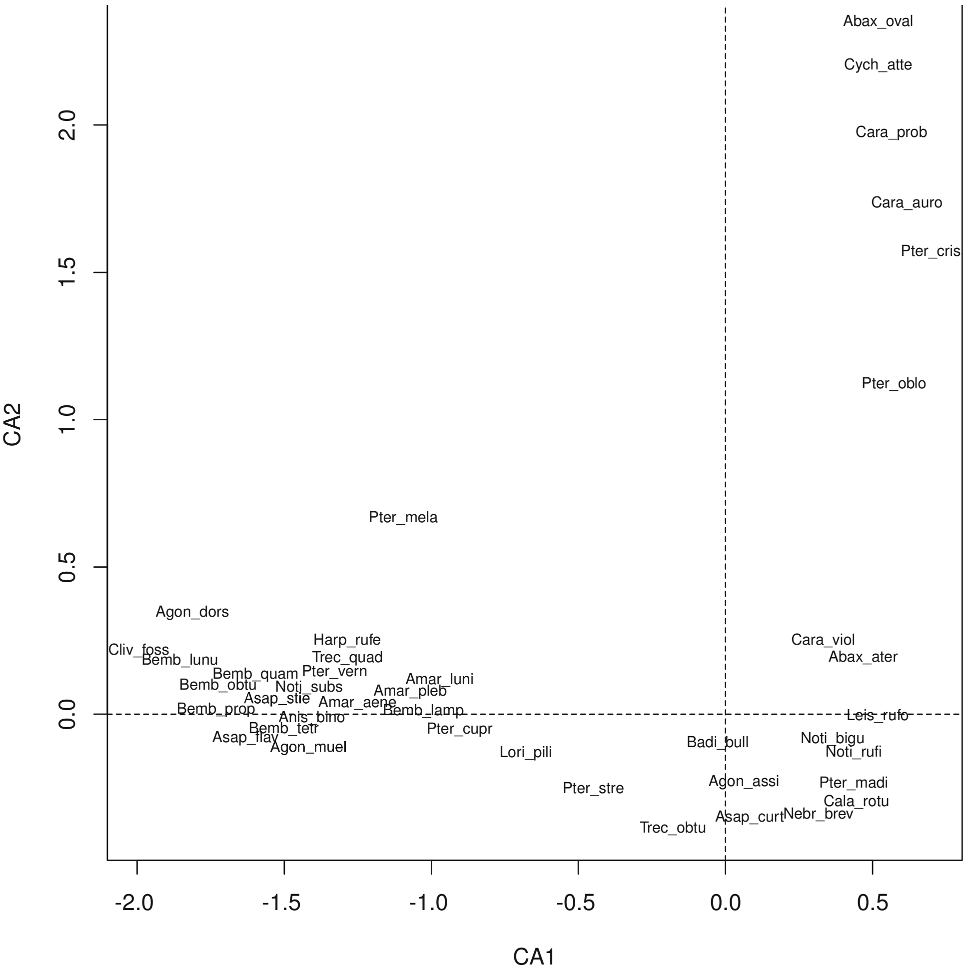

Fig. 2B - CA species plot: eigenvalue axis 1 = 0.514, eigenvalue axis 2 = 0.272. Species abbreviations are explained in Appendix 3.

The importance of fragment area and distance along the transect in structuring total species composition was further confirmed by a constrained CCA analysis with transect distance, fragment area and their interaction as explanatory variables. These three variables explained 33.5% of the total variation in species composition (Table 2, Fig 3). Permutation tests revealed a highly significant effect of the main effects (i.e. fragment area and distance along the transect) (Table 2). However, the effect of distance along the transect differed only slightly, but significantly, among fragments of different size.

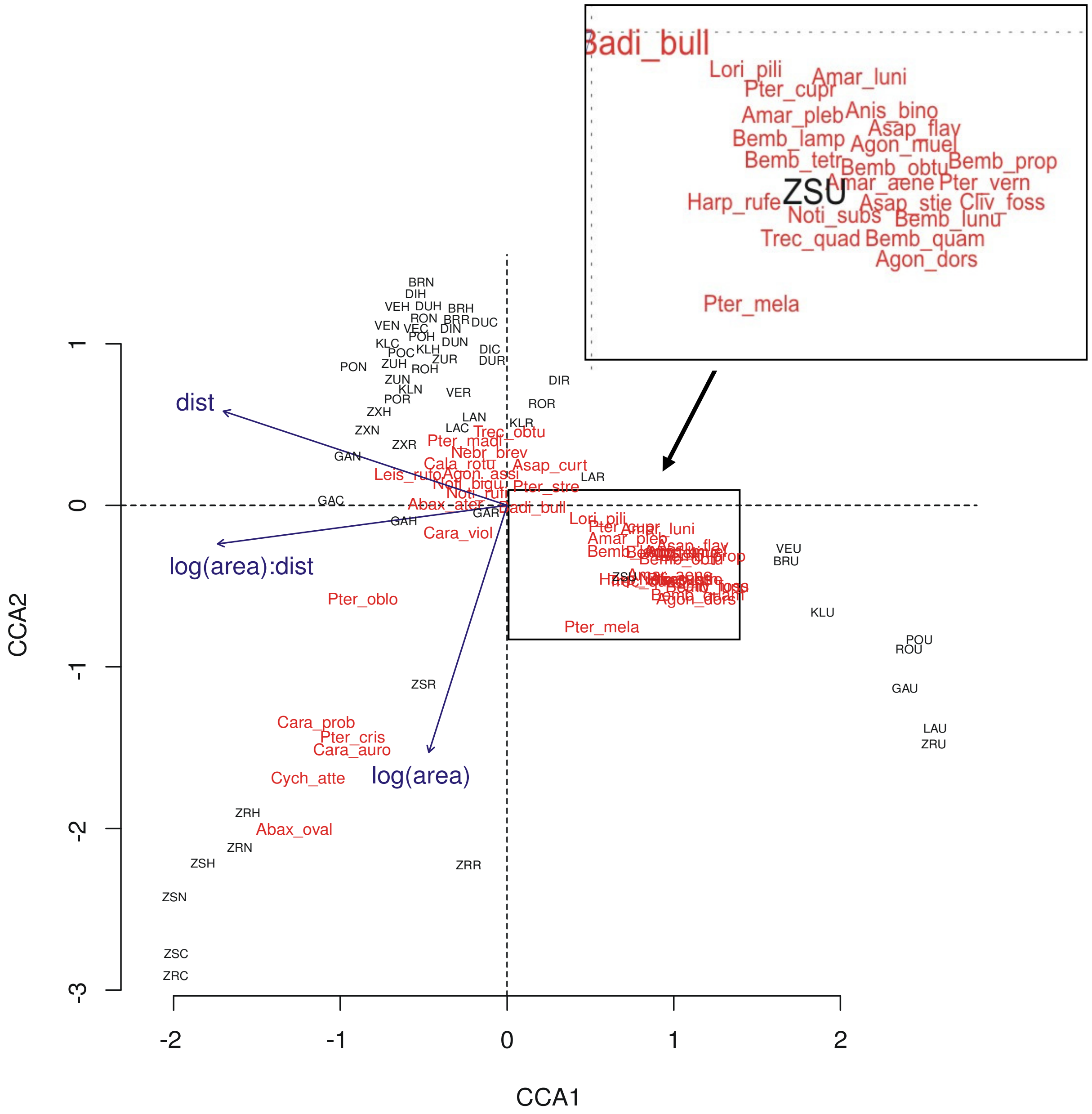

Fig. 3 - CCA triplot: eigenvalue axis 1 = 0.292, eigenvalue axis 2 = 0.220, with plots, species and environmental variables (fragment area, distance along the transect, and their interaction). Species abbreviations are explained in Appendix 3.

Table 2 - Results of the constrained analysis (CCA) including all the sample plots

CONSTRAINED ANALYSIS: all sample plots				
	Inertia	Proportion	Rank	
Total	1.5755	1.0		
Constrained	0.5285	0.3355	3	
Unconstrained	1.0470	0.6645	40	
Eigenvalus for constrained axes				
CCA 1	CCA 2	CCA 3		
0.29202	0.21972	0.01676		
Permutation test				
	Df	Chi sq	F	P
DIST	1	0.2719	12.4108	0.001
LN(AREA)	1	0.1810	8.2620	0.001
DIST:LN(AREA)	1	0.0646	2.9506	0.028
Residual	53	1.1612		

Given that the total variation in species composition is mainly driven by the large assembly differences of plots outside the forest, a second analysis was performed on a restricted dataset with the exterior plots removed, allowing us to better quantify information on edge effects within forest fragments. A constrained analysis on this restricted dataset (total inertia = 0.85) revealed that only fragment area had a significant effect on species composition, but neither the location of the plot along the transect or the interaction between distance along the transect and fragment area (Table 3). Hence, no consistent shifts in species assemblages were observed that discriminate plots situated closer to the edge compared to plots situated at the interior of the forest fragment.

Table 3 - Results of the constrained analysis (CCA) with exclusion of the plots located outside the first (-30)

CONSTRAINED ANALYSIS: outside plots removed				
	Inertia	Proportion	Rank	
Total	0.8499	1.0		
Constrained	0.2847	0.3350	3	
Unconstrained	0.5651	0.6650	21	
Eigenvalues for constrained axes				
CCA 1	CCA 2	CCA 3		
0.255629	0.021051	0.008069		
Permutation test				
	Df	Chi sq	F	P
Log (area)	1	0.2479	18.8588	0.001
Distance	1	0.0197	1.5001	0.133
Log (area):dist	1	0.0172	1.3073	0.202
Residual	43	0.5651		

Species turnover along gradients

The full model relating species turnover to all explanatory variables and their interactions revealed no significant interaction effect of fragment area with distance along the transect, indicating that the response of species turnover along the transects did not differ significantly between larger and smaller fragments (Table 4). After removing these effects in a stepwise manner, a final model was constructed including only the main effects being distance along the transect, a quadratic effect of distance and an LN(AREA) effect. Based on this model, only the effect of distance along the transect appeared to be significant, and indicated that the rate of species turnover compared to the plots outside the forest, decreased non-linearly from 0.77 at the forest edge to 0.87 at a distance of 100 m inside the forest (Fig 4).

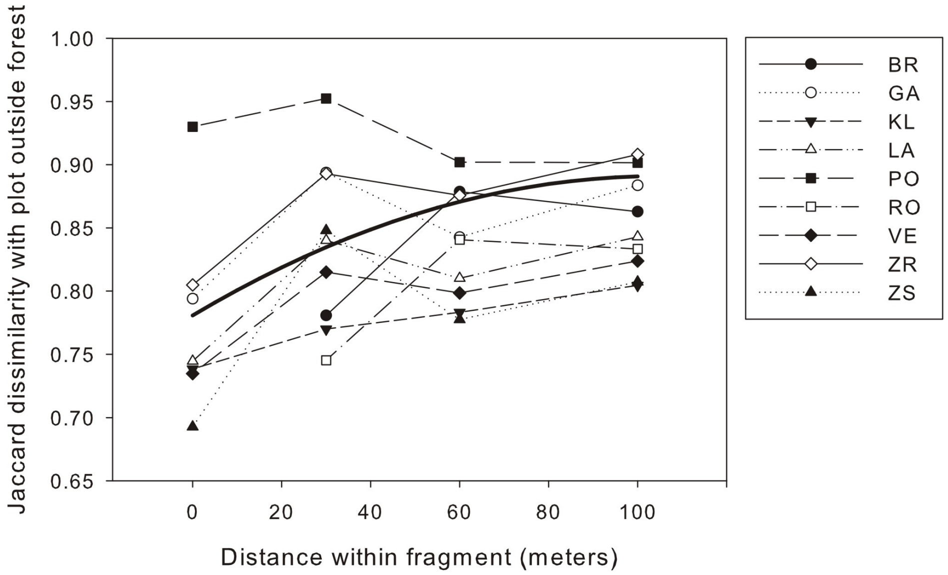

Fig. 4 - Pairwise Jaccard dissimilarity values calculated between the plots outside the forest (as reference) and all other forest plots along each transect. The bold line represents the predicted values as calculated from the General Linear Mixed model.

Table 4 - Results of the General Linear Mixed model between species turnover (Jaccard dissimilarities) and the explanatory variables distance along the transect (Dist), ln area (Area) and the quadratic distance effect. The interaction effects [distance*area] and [distance*distance*area] had no significant effect and were removed from the final model.

General Linear Mixed model

	df	Df	F	P	estimate	SE
Int					0.78	0.022
DIST	1	23.1	15.78	0.006	0.0021	0.0005
AREA	1	7.02	0.01	0.9	-9.99E-07	9.48E-06
DIST*DIST	1	23.2	8.15	0.009	-0.00001	5.06E-06
[DIST*AREA]	1	22.2	0.72	0.4		
[DIST*DIST*AREA]	1	21.2	0.3	0.6		

3.5 Discussion

Our results show that fragment area, rather than distance-to-edge is the most important variable shaping carabid assemblages within our studied forests. Changes in species composition in fragmented patches are most often explained by the replacement of habitat specialists that are bound to the interior of the patch with species residing in the matrix habitat (Halme & Niemelä 1993, Ås 1999, Magura et al. 2001, Summerville 2004, Lövei & Sunderland 2006, Didham et al. 2007, Ewers et al. 2007, Hendrickx et al. 2009, Magura 2010). Here, we explicitly tested this phenomenon by comparing differences in species composition between forest interior and matrix habitat for forests of different size. Our results, however, do not corroborate such an effect. Rather, the beetle community found at the edge of the forest was more similar to that of the forest interior than to assemblages typifying the surrounding matrix. Moreover, a similar beetle community was found, from the border of the forest to the interior, with only slight, though significant, edge effects. Notwithstanding, plots situated outside the forest appeared to be highly distinct from the species assemblages found in both larger as well as smaller forest fragments. This general pattern is in accordance with several studies on arthropod communities in forests, where a typical forest fauna is observed even at a close distance to the edge (Martin & Major 2001, Heliölä et al. 2001, Taboada et al. 2004, Basset et al. 2008). As indicated by the unconstrained CA analysis, the small differences in species composition between forest interior plots and those situated at the edge are most likely due to matrix species residing in the forest border rather than true edge preferring species (Magura et al. 2001, Molnar et al. 2001, Loveï et al. 2006).

Hence, given that edge effects appeared to be restricted to plots situated at the forest edge, it is remarkable that beetle communities from small forest fragments lack highly specialized species such as *Abax ovalis*, *Carabus problematicus*, *Carabus auronitens*, *Pterostichus cristatus* and *Cychrus attenuates*. Similar results were found by Gibb & Hochuli (2002). Moreover, forest area appeared to be the most important variable explaining differences in species composition among all plots located within the forest fragments.

Given that all studied fragments are very similar in habitat, soil and forest structure, and that edge effects appear to be restricted to the forest edge, local extinction of true forest specialist due to demographic and environmental stochastic effects are the most likely cause of the strong association between forest area and species composition. All mentioned forest specialists indeed appeared to be highly bound to core forest habitat and lack functional wings (Turin 2000) rendering them very unlikely to recolonize vacant forest fragments through the matrix after local extinction events (Hanski 1998, Roland et al. 2000, Keller et al. 2003). Generalist species, on the other hand, can clearly cope far better with habitat fragmentation than specialist species (Didham et al. 1996). Populations from large and continuous forests, then again, are less prone to local extinction as demographic and environmental fluctuations are less likely to completely result in population extinction.

The importance of local extinction followed by a lack of recolonization was also confirmed in a previous population genetic study conducted on the forest specialist *Carabus problematicus* where we showed not only strong effects of forest isolation on population isolation, but also a significant lower genetic diversity indicating larger stochastic effects (chapter 6).

That smaller forests suffer more from edge effects because of the higher ratio of edge per unit area, is another widely accepted phenomenon (Didham et al. 1998 a, b, Barbosa & Marquet 2002, Ewers et al. 2007). However, our CCA analysis with only plots from inside the forest, demonstrates the strength of edge effects to be independent from increasing habitat area.

Implications for conservation

We demonstrated that fragmentation in the forests around Brussels, causes species extinctions mainly by decreasing the amount of viable core habitat area and as such population sizes, rather than due to the often suggested increasing edge effects (cfr. Ewers et al. (2007)). Despite the urban character of the matrix surrounding the large ancient Soignes forest, it seems to contain old forest species even close to the edge of the forest. Hence, this suggests that the most important measure to preserve this typical fauna is to retain or enlarge forest fragments as large as possible such that they can serve as a sustainable refuge for specialized and unique species. In an urban environment, there is a clear segregation between forest and surrounding matrix. But, since these sharp edges had an equal influence on beetle communities in small and large fragments, even smaller forest stands may still be valuable and important for conservation, in particular when corridors among these fragments can be developed.

3.6 Acknowledgements

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3.8 Appendix

Appendix 3 – Species codes.

Species	Abbreviation	Species	Abbreviation
Abax ater	Abax_ater	*Carabus problematicus*	Cara_prob
Abax ovalis	Abax_oval	*Carabus violaceus*	Cara_viol
Abax parallelus	Abax_para	*Clivina collaris*	Cliv_coll
Acupalpus meridianus	Acup_meri	*Clivina fossor*	Cliv_foss
Agonum albipes	Agon_albi	*Cychrus attenuatus*	Cych_atte
Agonum assimile	Agon_assi	*Demetrias atricapillus*	Deme_atri
Agonum dorsalis	Agon_dors	*Diachromus germanus*	Diac_germ
Agonum muelleri	Agon_muel	*Dromius quadrimaculatus*	Drom_quam
Agonum nigrum	Agon_nigr	*Dyschirius globosus*	Dysc_glob
Agonum obscurum	Agon_obsc	*Dyschirius intermedius*	Dysc_inte
Agonum sexpunctatum	Agon_sexp	*Dyschirius politus*	Dysc_poli
Amara aenea	Amar_aene	*Dyschirius thoracicus*	Dysc_thor
Amara anthobia	Amar_anth	*Elaphrus cupreus*	Elap_cupr
Amara convexior	Amar_cono	*Harpalus affinis*	Harp_affi
Amara consularis	Amar_cons	*Harpalus distinguendus*	Harp_dist
Amara curta	Amar_curt	*Harpalus latus*	Harp_latu
Amara eurynota	Amar_eury	*Harpalus rufipes*	Harp_rufe
Amara familiaris	Amar_fami	*Leistus ferrugineus*	Leis_ferr
Amar lunicollis	Amar_luni	*Leistus fulvibarbis*	Leis_fulv
Amara ovata	Amar_ovat	*Leistus rufomarginatus*	Leis_rufo
Amara plebeja	Amar_pleb	*Loricera pilicornis*	Lori_pili
Amara similata	Amar_simi	*Metabletus foveatus*	Meta_fove
Amara tibia	Amar_tibi	*Molops piceus*	Molo_pice
Anisodactylus binotatus	Anis_bino	*Nebria brevicollis*	Nebr_brev
Asaphidion curtum	Asap_curt	*Nebria salina*	Nebr_Sali
Asaphidion flavipes	Asap_flav	*Notiophilus biguttatus*	Noti_bigu
Asaphidion pallipes	Asap_pall	*Notiophillus palustris*	Noti_palu
Asaphidion stierlini	Asap_stie	*Notiphillus quadripunctatus*	Noti_quad
Badister bullatus	Badi_bull	*Notiophillus rufipes*	Noti_rufi
Badister lacertosus	Badi_lace	*Notiophillus substriatus*	Noti_subs
Badister sodalis	Badi_soda	*Parophonus maculicornis*	Paro_macu
Bembidion biguttatum	Bemb_bigu	*Patrobus atrorufus*	Patr_atro
Bembidion deletum	Bemb_dele	*Pterostichus anthobia*	Pter_anth
Bembidion femoratum	Bemb_femo	*Pterostichus cristatus*	Pter_cris
Bembidion harpaloides	Bemb_harp	*Pterostichus cupreus*	Pter_cupr
Bembidion lampros	Bemb_lamp	*Pterostichus madidus*	Pter_madi
Bembidion lunulatum	Bemb_lunu	*Pterostichus melanarius*	Pter_mela
Bembidion obtusum	Bemb_obtu	*Pterostichus minor*	Pter_mino
Bembidion properans	Bemb_prop	*Pterostichus niger*	Pter_nige
Bembidion quadrimaculatum	Bemb_quam	*Pterostichus nigrita*	Pter_nigr
Bembidion tetracolum	Bemb_tetr	*Pterostichus oblongopunctatus*	Pter_oblo
Bradycellus harpalinus	Brad_harp	*Pterostichus strenuus*	Pter_stre
Bradycellus rufipes	Brad_rufi	*Pterostichus vernalis*	Pter_vern
Bradycellus verbasci	Brad_verb	*Pterostichus versicolor*	Pter_vers
Calathus fuscipes	Cala_fusc	*Stenolophus teutonus*	Sten_teut
Calathus melanocephalus	Cala_mela	*Synuchus nivalis*	Synu_niva
Calathus rotundicollis	Cala_rotu	*Trechoblemus micros*	Trec_micr
Carabus auronitens	Cara_auro	*Trechus obtusus*	Trec_obtu
Carabus monilis	Cara_moni	*Trechus quadristriatus*	Trec_quad
Carabus nemoralis	Cara_nemo	*Trichotichnus laevicollis*	Tric_laev

Fish eye photo Soignes forest © Eva Gaublomme

Chapter 4

ISOLATION AND CHARACTERIZATION OF MICROSATELLITE LOCI IN THE GROUND BEETLE *CARABUS PROBLEMATICUS* (COLEOPTERA, CARABIDAE)

Chapter 4

Isolation and characterization of microsatellite loci in the ground beetle *Carabus problematicus* (Coleoptera, Carabidae)

EVA GAUBLOMME, HILDE DHUYVETTER, PETER VERDYCK, GUENAELLE MONDOR-GENSON,
JEAN-YVES RASPLUS AND KONJEV DESENDER

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4.1 Summary

Six microsatellite loci were isolated from the ground beetle *Carabus problematicus.* Polymorphism ranges from 2 to 17 alleles, and observed and expected heterozygosities range from 0.137 to 0.676 and 0.147 to 0.710 respectively.

Keywords: Carabidae, Carabus problematicus, Coleoptera, genetic diversity, ground beetle, microsatellites

4.2 Methods and results

Carabus problematicus is one of the three most common *Carabus* species in Europe (Turin, 2000). This large wingless ground beetle occurs in Western Europe from Finland to southern France, including the British Isles. The species shows a variable habitat preference according to the region. From southern France to Belgium, it prefers old forests, while to the North and at higher elevation it is increasingly found in open heath like habitats. Allozymes have been used in population genetic studies on the effects of habitat fragmentation and history (Desender et al., unpublished data), but their resolution is insufficient at a small geographic scale. Microsatellite DNA is an appropriate marker for quantifying genetic differentiation at a fine temporal and spatial scale. The extra sensitivity inherent to potentially hyper variable microsatellite loci (Tautz 1989) clearly facilitates genetic analysis of populations.

A microsatellite enriched genomic library was built as described in Dutech et al. (2000) using biotin-labelled microsatellite oligoprobes and streptavidin-coated magnetic beads. Minor modifications were: (i) isolation of 300-900bp fragments from *RSA*I-digested genomic DNA; (ii) ligation of Biotin selected genomic fragments into pGEM®-T Easy cloning vector (Promega) for transformation of JM109 competent cells (Promega); (iii) screening of recombinant colonies with repeat-containing oligonucleotides by PCR in a 10-µL final volume (MJ Research PTC 100 termal cycler). Each reaction mix contained 1 µL of plasmid, 1x buffer (Promega, PCR Core System I, thermophilic DNA Polymerase 10x Reaction Buffer, $MgCl_2$-Free), 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.1 U of Taq polymerase (Promega) and 1 µM of each primer (one primer for the vector (SP6 or T7) and a second repeat-containing oligonucleotide: $(CT)_{10}$, $(TG)_{10}$, $(CT)(ATCT)_6$, $(TGTA)_6(TG)$, $(TAA)_8$ or $(GAA)_8$). Reaction conditions were as follows: an initial denaturation step of 3 min at 95°C, 24 cycles of 40 s at 94°C, 60 s at 58°C and 2 min at 72°C. Extra amplification products were detected in 100 clones and sequenced by Eurogentec (Seraing, Belgium).

Primers were designed for 22 microsatellite-bearing clones using Primer 3 web-based software package (Rozen & Skaletsky 1998) and tested on DNA, extracted with the Qiamp DNA mini kit (Qiagen). DNA from each individual was taken from muscles of the femur or from a small (4-5 µL) *in vivo*-obtained haemolymph sample (Gaublomme et al. 2002). Microsatellite DNA amplification reactions were performed in a 10 µL final volume (Biometra T gradient cycler). Each reaction mix contained 1 µL of extract (approximately 10 ng of DNA), 1x buffer (Qiagen, 10x concentrated, contains Tris Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$; pH 8.7), 200 µM of each dNTP, 0.4 µM of each primer (forward primer Cy5 labelled) and 0.3 U hotStarTaq DNA polymerase (Qiagen). For all loci, PCR amplifications were carried out using a Biometra T personal cycler. Following parameters were used: 15 min at 94°C for one cycle, followed by 30 cycles of 94°C for 45 s, annealing temperature (Table 1) for 45 s and 72°C for 60 s, followed by one cycle of 5 min at 72°C. Amplification products were scored on an ALF Express sequencer (Amersham Pharmacia Biotech, 6% polyacrylamide gel), and product sizes were determined by comparison with M13mp8 DNA standards, following van Oppen et al. (1997). Out of 22 primer pairs tested, six gave a clear polymorphic banding pattern. We analysed individuals from 10 populations from Belgium. Observed and expected heterozygosities were calculated using GENEPOP (Raymond & Rousset 1995). Exact tests for deviations from Hardy-Weinberg equilibrium and from linkage disequilibrium were also calculated in GENEPOP for four loci, but yielded no deviations. For Cpro55 and Cpro 99 these tests were not performed because the 32 to 40 individuals came from 8 different populations and equilibrium could not yet be tested. The number of alleles for the six loci ranged from 2 to 17 and the observed and expected heterozygosities from 0.137 to 0.676 and 0.147 to 0.710, respectively. Additionally, we tested the amplification of these primers in minimum 8 specimens of each of the related species: *C. nemoralis, C. violaceus* and *C. auronitens.* (Table 2). Some of the markers, developed for *C. problematicus,* cross-amplified in the related species, but only Cpro 98 yielded interpretable amplified products.

Table 1 - Characterisation of six *Carabus problematicus* microsatellite loci. ‘Size’ refers to the length (in bp) of the cloned allele; ‘T_a’: annealing temperature in °C. Number of alleles, observed heterozygosity H_O, expected heterozygosity H_E are given for all screened individuals**.**

Locus	Repeat motif	Primers (5’ to 3’)	T_a	$MgCl_2$	Size	Number of alleles	Number of analysed specimen	H_O	H_E	Accession no.
Cpro40	CACA(TACA)TCA$(TACA)_4$	F: TGCGCAGACGTTGTAATTTG R: GGAGCGCAGCCTGTATGTAT	57,6	1	200	3	309	0,465	0,474	AY191514
Cpro44	$(TACA)_2(CA)_2$ $(TACA)_2$GACA	F: TAGCTTCCCTCCGTTCGTC R: TCCGTGGAATATTGGAAAGC	57,6	1,5	197	2	305	0,137	0,147	AY191515
Cpro55	$(CA)_6$	F: GGCAAGCAGGCAAGTAAGTT R: CAAGCATTCTACCTGGACTGAA	55,2	1,5	189	5	40	0,248	0,269	AY191516
Cpro97	$(CA)_7$	F: GCATGCACGCAGTATACACA R: ATAGACGCCTCGACAGGTTG	54	1	199	4	283	0,536	0,569	AY191517
Cpro98	CAA$(CA)_{11}$CG	F: CCCTAATCAGACGACGCAGT R: AAATTCAATTCCCGCAAGGT	60,2	1,5	190	17	283	0,676	0,710	AY196360
Cpro99	$(AC)_7$	F: GTTCGCTCCTGATGGAAATG R: GGGAGTGTAGCAATGCCAAT	57,6	1	179	11	32	0,203	0,630	AY191518

Table 2 Cross species amplification of *Carabus problematicus*-markers in other *Carabus* species (*C. nemoralis*, *C. violaceus* and *C. auronitens*)

(+) interpretable amplification in all individuals tested, (±) suboptimal amplification, (-) no product was amplified

	Cpro40	Cpro44	Cpro55	Cpro97	Cpro98	Cpro99
Carabus auronitens	-	-	±	±	+	-
Carabus violaceus	-	±	±	±	+	-
Carabus nemoralis	-	-	±	±	+	-

4.3 Acknowledgments

This work is financially supported by the Entomology Department of the Royal Belgian Institute of Natural Sciences, project DWTC2202DES3, and is carried out within the framework of the Flemish research network FWO.010.97N (‘Ecological genetics: patterns and processes of genetic variation in natural populations’). Also sincere thanks to the staff of I.N.R.A.- C.B.G.P. (Montferrier-sur-Lez) for their help during our stay in their laboratory.

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Dielegem forest © Eva Gaublomme

Chapter 5

NON-DESTRUCTIVE SAMPLING FOR GENETIC STUDIES ON *CARABUS AURONITENS* AND *CARABUS PROBLEMATICUS*: A STUDY BASED ON ALLOZYMES AND MICROSATELLITES.

Chapter 5

Non-destructive sampling for genetic studies on *Carabus auronitens* and *Carabus problematicus*: A study based on allozymes and microsatellites

EVA GAUBLOMME, KONJEV DESENDER, PETER VERDYCK, HILDE DHUYVETTER AND JEAN-YVES RASPLUS

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5.1 Summary

The development of a non-destructive method for detailed conservation genetic research is urgent for the ecological genetic monitoring of remnant populations. We therefore developed and tested a technique to study the relatively large beetle species *Carabus auronitens* and *Carabus problematicus* with allozymes and microsatellite markers. We used in vivo obtained tissue or haemolymph samples and aimed at minimally influencing animal survival. The non-destructive technique, as being presented here, offers applications at different spatial scales from forest fragments up to inter regional level.

Keywords: *Carabus auronitens*, *Carabus problematicus*, microsatellites, allozymes, haemolymph, tissue sample, survival after sampling

5.2 Introduction

In Flanders many populations of terrestrial arthropods survive in remnants of their natural habitat. They are isolated by an intensively cultivated landscape (Kuijken 1999). Habitat fragmentation (i.e. loss of habitat, reduced patch size and increasing isolation) results in reduced biodiversity (Andren 1994), but can also lead to a higher genetic differentiation as a consequence of reduced gene flow (Hastings & Harrison 1994, Young et al. 1996).

Fragmentation is supposed to have a negative effect on biodiversity caused by increased genetic erosion and consequently a lower adaptation ability. The decrease in genetic variation can eventually lead to a higher extinction rate of populations (Frankham 1995).

Woodlands in Flanders belong to the most fragmented forests in Western Europe. Several flightless ground beetles of the genus *Carabus* appear to be excellent indicator species of ancient forests in this region (Desender et al. 1999). Previous research on the population genetic consequences of habitat fragmentation for population genetics in function of policy recommendations indicates that for invertebrates more data are needed on rarer (threatened) model species. The development of a non-destructive method for detailed conservation genetic research is relevant for ecologically justified genetic monitoring of remnant populations or populations after potential colonization and/or reintroduction. We make use of invertebrates as key species.

In this contribution, we therefore develop and test a technique to study the relatively large beetle species *Carabus auronitens* and *Carabus problematicus,* using only very small *in vivo* obtained tissue or haemolymph samples and aiming at minimally influencing animal survival. We investigate survival after leg tissue and haemolymph sampling and test the biochemical performance of the samples for allozyme electrophoresis and microsatellite marker after amplification.

5.3 Material and methods

C. *auronitens* is a European ground beetle species. In Belgium it is widespread in the South-East (Desender, 1986) and rare in Flanders (Desender et al., 1995). It is a stenotopic species that prefers relatively humid and deciduous forests.

C. *problematicus* is also a European species with an Atlantic Middle European distribution. This species is widespread in the southern part of Belgium and restricted to old forests.

Beetles were collected during overwintering[2] in Autumn 2000 by hand catches. We conducted a standardized survival experiment for about 8 weeks and compared the performance of large series of beetles in three categories. For each species, a total of 120 individuals, was divided into three equal groups. The first group was left untreated, from individuals of the second group one middle leg was cut of just underneath the coxa and from individuals of the third group a small haemolymph sample varying from 3 up to 10 µl was taken. This haemolymph was obtained by making an intrusion in the metepisternum using a small capillary. Samples were kept in a freezer. All treated individuals were kept between mosses in outdoor winter conditions in temperatures varying from 4 to 10°C, without food. For 57 days survival was checked almost daily.

Cellulose acetate electrophoresis was used for screening 6 polymorphic loci for C. *auronitens* and 7 polymorphic loci for C. *problematicus.* The technique used is described in Hebert & Beaton (1989). Banding patterns obtained from tissue samples were compared to those obtained from haemolymph samples. Tissue samples (one leg in 30 µl distilled water) and haemolymph samples (3-4 µl, diluted up to 10 µl) from five individuals each were compared. This way we can deduce if equal patterns are obtained for both types of samples and/or if stronger or weaker bandage patterns appear on gel. Muscle tissue, taken from one leg, is normally sufficient for the simultaneous and straightforward study of a number of allozyme loci as well as microsatellite loci in these species.

To perform screening of microsatellite loci (technique following Estoup et al. 1993), DNA was extracted from haemolymph samples as well as from tissue samples and was tested for four microsatellite loci. Primers were developed and optimized for the species C. *auronitens* and preliminary tested under the same conditions for C. *problematicus.* For the extraction of both sample types, we used a Qiaamp (Qiagen) extraction kit. Screening was performed using automatic ALF Express sequencers (Amersham Pharmacia biotech, 6% poly-acrylamide gels).

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[2] Sampling was conducted during winter, but this was only relevant for the current study. Non-destructive sampling is most probably better during their main activity period, which has probably less implications for the survival of the individuals when released in their natural environment.

5.4 Results and conclusions

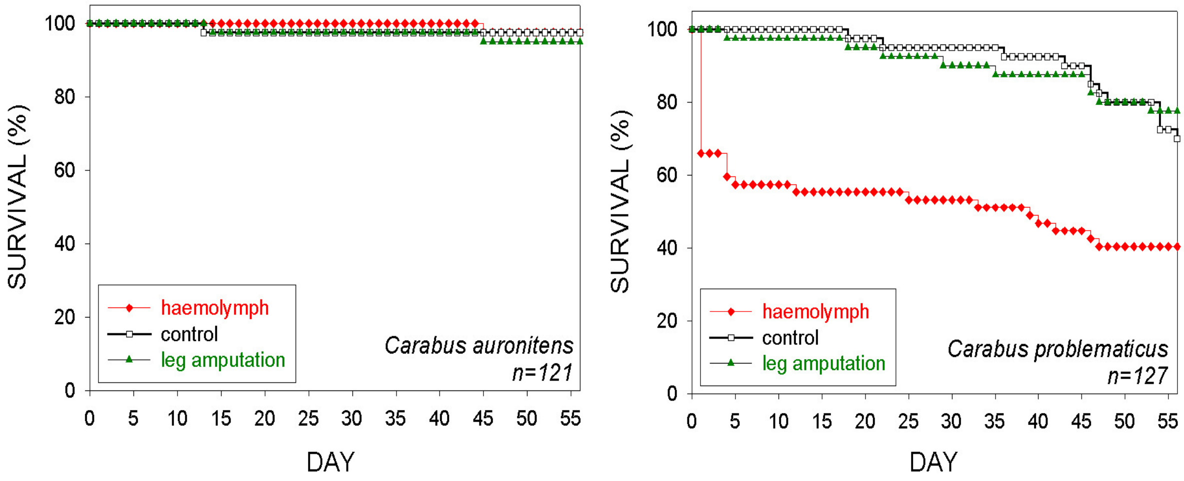

Fig. 1 - Survival curves for *C. auronitens* and *C. problematicus* after taking a haemolymph sample and the amputation of a middle leg, compared to a control group.

Survival after sampling

By comparing the effects of leg amputation or haemolymph sampling between the two species, we found a significant difference for haemolymph sampling ($\chi2 = 32.35$ and $p = 0.0000$). We noticed a higher initial mortality for *C. problematicus,* but not for *C. auronitens* (Fig 1). Mortality should thus be studied for each species separately and may not be generalized for all Carabidae. The higher mortality rate could be due to the more intrusive character of the manipulation combined with a softer cuticle.

Physiological research by Remmer (1982) using haemolymph suggested that there was no higher mortality for *Carabus* species by taking a small haemolymph sample. This investigation however was based only on 10 specimen which were fed. In our experiment beetles were sampled during hibernation, when metabolism is probably low due to lack of food and low temperatures. So, beetles were kept in similar circumstances during the experiment and feeding was not necessary.

The amputation of one middle leg doesn't show clear differences between the survival of the two species. Remarkably, the amputation of one middle leg does not seem to increase mortality. This means that this technique is applicable to study rare or threatened species and might thus be compared to toe-clipping in Mammalia and finclipping in Pisces. Regular findings of beetles lacking one leg in the field further suggest this has indeed not a profound negative effect on short term survival.

Leg amputation was used already for other *Carabus* species and did not seem-to affect mortality rate or reproductional success (Rasplus - unpublished).

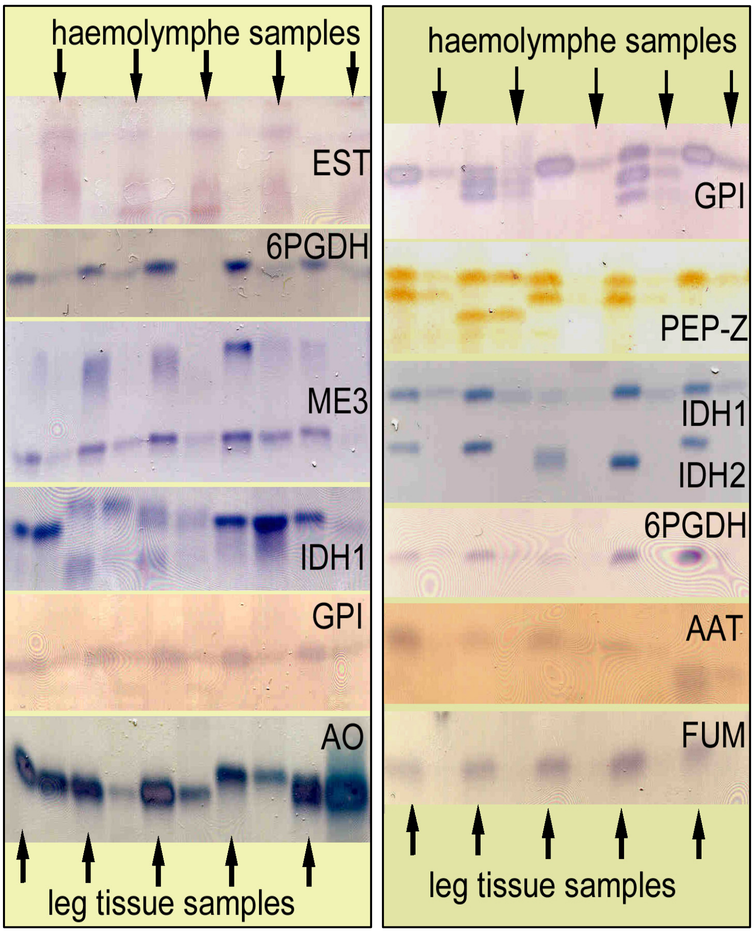

Fig. 2 - Comparison of cellulose acetate gels for the enzymes used for *C. auronitens* (left series) and *C. problematicus* (right series) for six and seven enzyme loci respectively.

Electrophoresis comparing haemolymph and tissue samples

Allozymograms (visualized by cellulose acetate electrophoresis, a technique only requiring very small samples) mostly can be derived from haemolymph samples, but yield patterns which are much more difficult to interpret than those derived from small tissue samples (Fig 2).

For C. *auronitens* we notice that bandage patterns are equally independently of this usage of haemolymph or tissue sample for the enzymes AO, GPI and IDH1. Much weaker patterns are observed for the enzymes ME3 and 6PGDH when using haemolymph. The interpretation, however, is identical. For EST we see a much clearer pattern when using haemolymph samples, but interpretation is difficult (this is often the case for esterase zymograms).

For C. *problematicus* haemolymph samples show weaker patterns for all allozymes, but are still interpretable and identical for the enzymes GPI, PEP-Z and IDHl, are much weaker with identical pattern or 6PGDH and much weaker with possibly another pattern for AAT. Bandage pattern is almost absent for FUM and IDH2. This last enzyme however is very variable and thus very informative when using tissue sample.

We can conclude that the use of one leg is much more efficient and gives better overall results. This is another argument to use a (middle) leg instead of the more time consuming haemolymph sampling, which additionally increases mortality in C. *problematicus.*

Microsatellite screening comparing haemolymph and tissue samples

Haemolymph samples are, in contradiction to their less efficient use in enzyme electrophoresis, very useful for the screening of microsatellites. Results indeed were equally clear when using haemolymph or tissue samples for all four markers (Fig 3).

5.5 Summary

Previous research on the population genetic consequences of habitat fragmentation indicates that for invertebrates more data are needed on rarer (threatened) model species. Therefore, non-destructive population genetic research and the development of powerful genetic markers are needed.

Results are given of a standardized survival experiment after obtaining *in vivo* tissue and haemolymph samples from the relatively large beetle species *Carabus auronitens* and *Carabus problematicus*. Mortality rates are extremely low, especially after amputation of one middle leg. The biochemical performance of the nondestructive technique is tested with allozymes and microsatellite markers.

Allozymograms show good staining results for haemolymph samples, but yield patterns which are much more difficult to interpret than those derived from small

tissue samples. Results from both types of samples are equally clear for microsatellite loci. Muscle tissue, taken from one leg, is sufficient for the simultaneous and straightforward study of a number of allozyme as well as microsatellite loci. The non-destructive technique, as presented here, offers applications at different spatial scales from forest fragments up to interregional level.

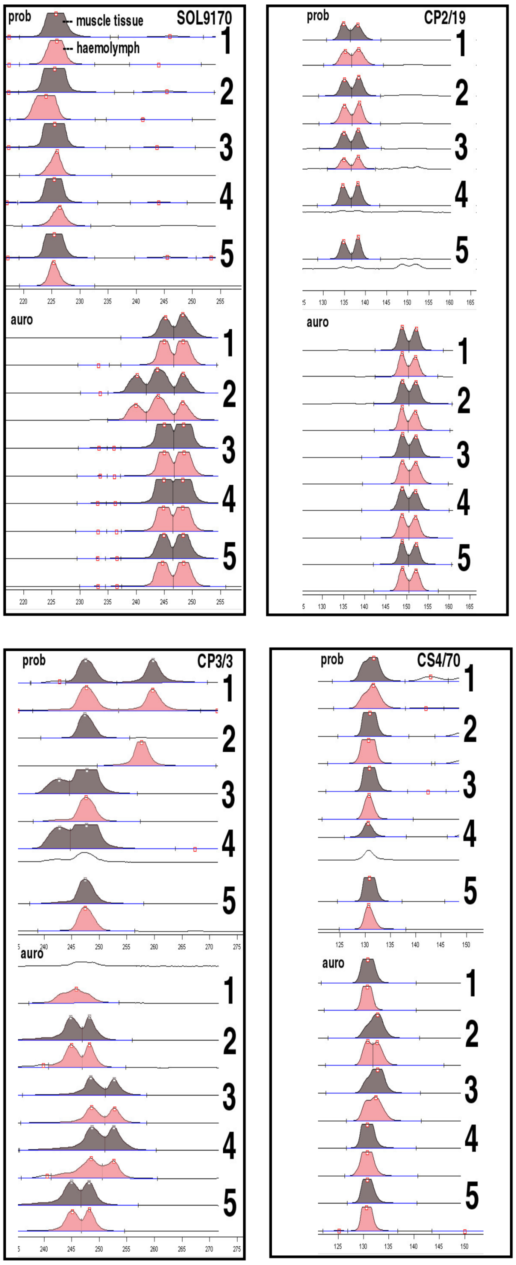

Fig. 3 - Microsatellite screening (automatic sequencer) for 5 specimens of *C. problematicus* (prob) and *C. auronitens* (auro), after PCR based on tissue sample (lst series, black filling of the sequencing peaks) and on haemolymph sample (2nd series) for 4 microsatellite-loci.

5.6 Acknowledgements

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Carabus problematicus © Jürgen Kottmann

Chapter 6

LOSS OF GENETIC DIVERSITY AND INCREASED GENETIC STRUCTURE IN RESPONSE TO FOREST AREA REDUCTION IN THE FLIGHTLESS CARABID BEETLE *CARABUS PROBLEMATICUS* (COLEOPTERA: CARABIDAE).

Chapter 6

Loss of genetic diversity and increased genetic structure in response to forest area reduction in the flightless carabid beetle *Carabus problematicus* (Coleoptera: Carabidae).

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Submitted to Insect Conservation and Diversity

6.1 Summary

1. The carabid fauna of ancient temperate broadleaved forests is characterized by a large proportion of forest specialists with low dispersal capability. Hence, species bound to this habitat are expected to be highly susceptible to the effects of decreasing patch size and increasing isolation.

2. Here, we investigate the relative effect of both factors by genotyping individuals of the flightless and forest specialist beetle *Carabus problematicus* from 29 populations, sampled in 21 different forest fragments in Belgium, at seven microsatellite loci.

3. A high degree of genetic differentiation among fragments was observed, with populations from smaller forests being considerably more differentiated and characterized by a lower genetic diversity compared to those of larger forests. A more detailed study on forest remnants of a former historic continuous woodland area revealed that population differentiation was significant among, but not within remnants, irrespective of geographic distance. This indicates that patch fragmentation rather than geographic distance is the ultimate factor that hampers gene flow in this species.

4. In summary, these results indicate that gene flow among suitable habitat patches is primarily reduced by the inability of this specialized species to traverse the landscape matrix. This lack of dispersal may pose a serious threat for the persistence of ecologically similar species, and present populations can best be protected by securing or increasing the size of existing habitat patches.

Keywords: microsatellites, ground beetle, population genetics, habitat fragmentation, isolation, genetic differentiation

6.2 Introduction

The fragmentation and destruction of natural habitats is a key threat to biodiversity and a major issue of conservation biology (Harris 1984, Meffe & Carroll 1997, Streiff et al. 2005). Fragmentation, deterioration and loss of habitat also threaten the survival of many insect species (Heisswolf et al. 2009), but effects will differ depending on the trophic level, dispersal ability and habitat specialization (Ewers & Didham, 2006) of the species under study. In particular, species with limited dispersal ability are expected to be the most vulnerable as metapopulation theory predicts that habitat size and isolation are the most critical factors determining the occurrence and survival of populations in a fragmented landscape (Leisnham & Jamieson 2002). If a formerly continuous population is divided into several isolated fragments, this reduces the effective size of the populations within each fragment (Frankham et al. 2002, Keller et al. 2005), which increases the effect of stochastic local extinctions. When dispersal is limited, recolonization of empty patches is strongly hampered and may ultimately result in the extinction of an entire metapopulation (Hanski 1998, Roland et al. 2000, Frankham et al. 2002).

A well-documented example of a landscape that suffered severe fragmentation are forest ecosystems in temperate Europe, which have been subjected to human influence for more than 7000 years (Verhulst 1995, Bloemers & Van Dorp 1991, Honnay et al. 2005). In Belgium, the transition of natural forests to agricultural land decreased forest cover by more than 70% (Bloemers & Van Dorp 1991, Tack et al. 1993, Tack & Hermy 1998). Many forest organisms have been highly affected by this reduction in forest area, and it most probably led to the extinction of several species at a national level (Tack et al. 1993, Ervynck et al. 1994, Bossuyt et al. 1999, Desender et al. 1999, Hermy et al. 1999, Honnay et al. 1999). Although habitat fragmentation is known to affect the long-term survival of many species, little information is available regarding its consequences on invertebrates from temperate woodlands. However, studies that investigated the population genetic effects of landscape fragmentation on ground beetle populations showed that landscape deterioration may strongly affect genetic structuring (Keller & Largiader 2003, Broaut et al. 2004, Garnier et al. 2004, Keller et al. 2004, Drees et al. 2008, Brouwers & Newton 2009, Matern et al. 2009). In particular, wingless and specialized species that are unlikely to find suitable land cover for dispersal among patches, are expected to be most vulnerable to the effects of increased patch isolation and decreasing habitat quality. Furthermore, the reduction in local effective population size may strongly increase genetic structure and result in an overall decrease in genetic variation (Frankham 1996). This loss of genetic variation may ultimately reduce the survival and fitness of affected populations (Frankham 1995, Ebert et al. 2002, Reed & Frankham 2003) and as such pose a threat to the long-term viability of populations (Frankham 1996, Saccheri et al. 1996, Saccheri et al. 1998, Westemeier et al. 1998, Desender et al. 1999).

Given that genetic differentiation among populations is supposed to be high in species with narrow ecological niches, low dispersal capability and a lack of behavioural response to habitat fragmentation (Wiens et al. 1997), species that share these characteristics are ideal to study the population genetic consequences of habitat fragmentation and to develop guidelines for the restoration of habitat remnants. Such restoration measures may include the creation of new habitat patches, the enlargement of existing ones or increasing the connectivity of existing patches in order to mitigate the effects of fragmentation. Especially within densely populated and urbanized areas, there is a strong need for such guidelines among policy makers, managers and conservation practitioners.

The carabid beetle *Carabus problematicus* occurs from Finland to Southern France, including the British Isles (Turin 2000), with Belgium situated in the centre of its distribution area. Because this species is a wingless ground beetle, long distance dispersal by flight is not possible, and it is therefore expected to suffer from isolation and fragmentation. Here we investigate the genetic structure of *C. problematicus* populations in a fragmented landscape and how this can be related to the factors forest area and geographical distance.

6.3 Material and methods

Study species

C. problematicus Herbst, 1786 is a strictly nocturnal beetle and an autumn breeder with larval hibernation and overwintering adults, thus having a two-year life cycle. It is feeding on snails and insects (Skuhravy 1959). The species is wingless, but movement up to 70-75 meters per night has been observed (Neumann 1971, Rijnsdorp 1980).

Study area and sampling

A total of 1221 beetles were sampled from 21 different forest fragments scattered throughout Belgium, varying in size and degree of isolation (Table 1, Fig 1). In some larger fragments that formerly belonged to the historic and continuous Kolenwoud forest (see below), multiple sites were sampled. Hence, the total sampling consisted of 29 different sites, hereafter called populations (Table 1). About 40 beetles per population were collected from May to September 2002 using live pitfall traps or by actively searching potential hibernation habitats during winter (e.g. bark of tree trunks). Traps were emptied every fortnight and up to 19 sampling campaigns were hold depending on the difficulty to reach a sampling of 40 individuals.

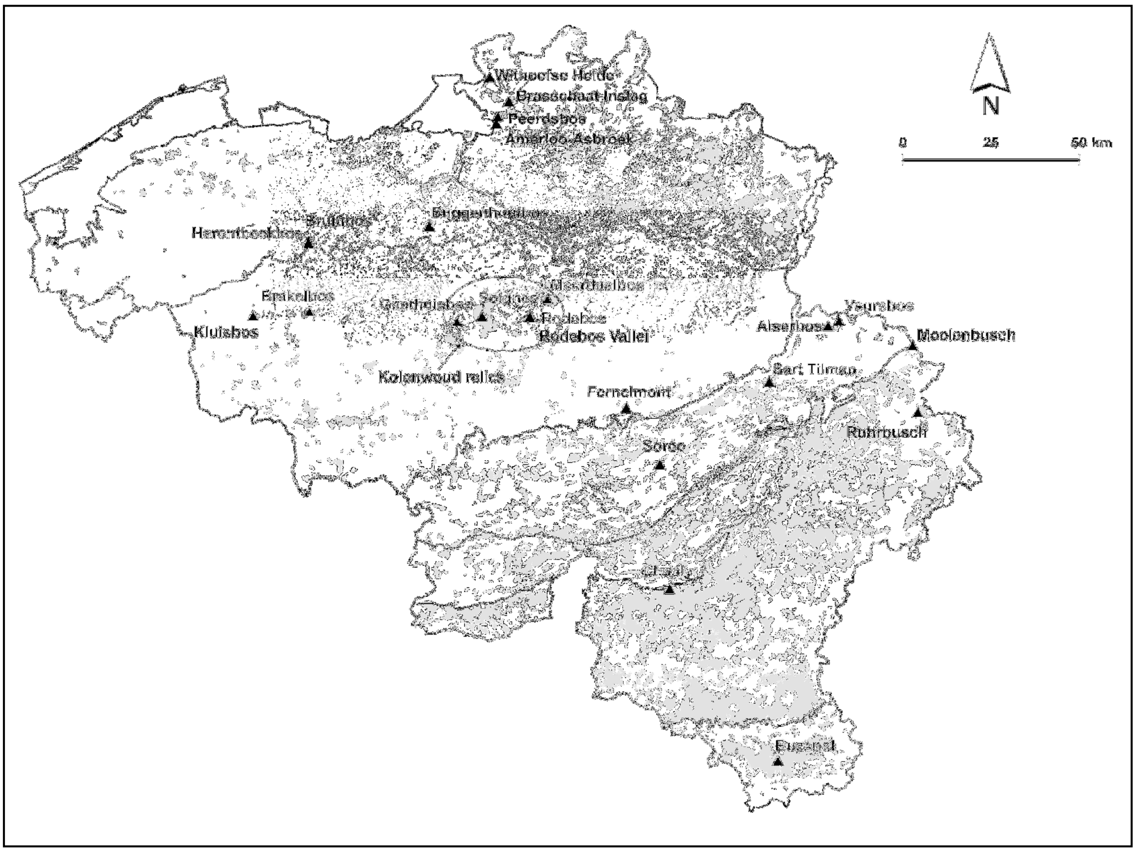


Fig. 1 - Geographic distribution of studied *Carabus problematicus* populations in Belgium. Forests are indicated in grey and sampling locations with triangles. The black lines represent Belgian ecoregions.

Of particular interest was the Brussels Capital Region, with a range of four ancient forest fragments: Gasthuisbos, Rodebos, Meerdaalwoud and Soignes (Fig 2). The historical ecology of these latter forests is well documented (Verhulst 1995, Van der Ben 1992, Tack et al. 1993). All fragments originated from the historic forest Kolenwoud (Table 1, Fig 1), which was one of the largest Belgian forests in the 10th century. A thorough sampling on a smaller scale was conducted here, wherein several populations per fragment were sampled: Soignes (n=2), Gasthuisbos (n=1), Meerdaal (n=7) and Rodebos (n=2; Table 1, Fig 2). This detailed sampling in the Brussels Capital Region most probably comprises all current Kolenwoud remnants wherein the study species is still present and as such reflects its current distribution there.

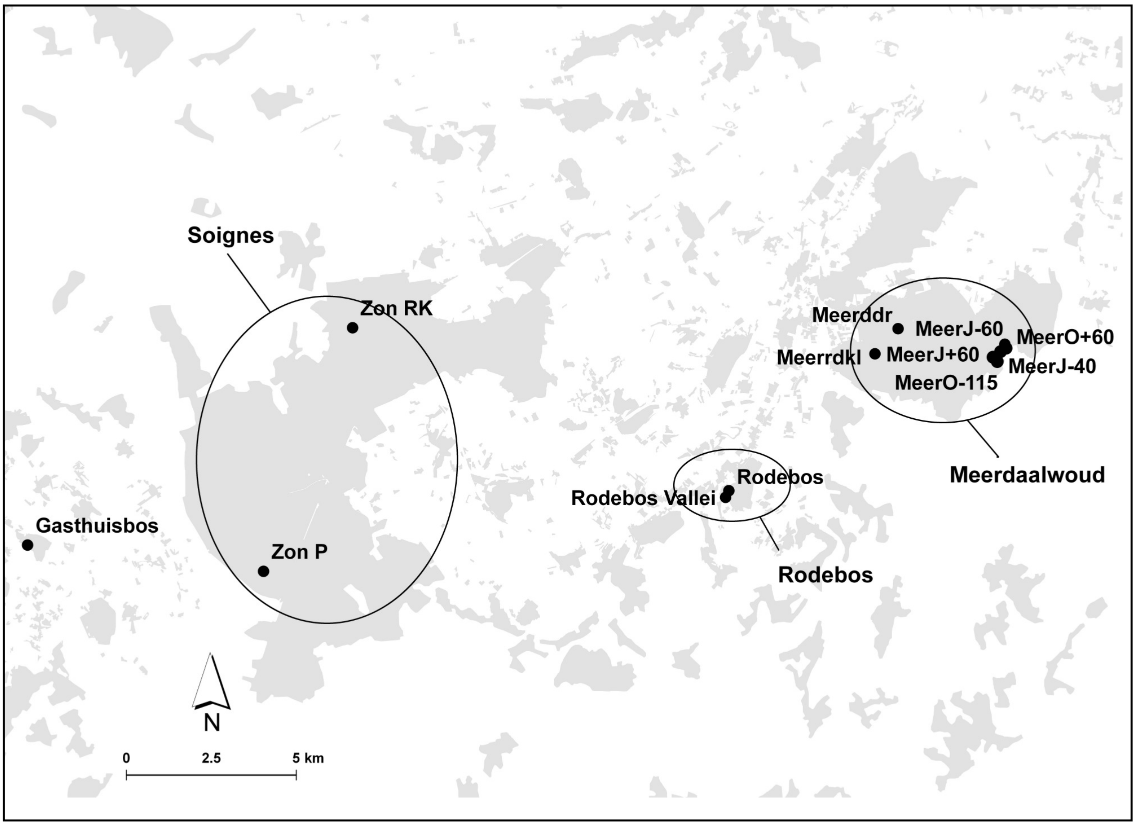


Fig. 2 - Detail of the four Kolenwoud fragments with twelve sampled populations of *Carabus problematicus*. For position and location see Fig 1.

Microsatellite genotyping

A total of eight microsatellite markers was used to genotype each individual. The loci P44, P40, P97, P98, P55, P99 have previously been isolated from *C. problematicus* (Gaublomme et al. 2003), the other two loci, CV105136CMPG and CV104481CMPG, were selected from *C. violaceus*, Linnaeus 1758 (Keller & Largiadèr 2002, Keller Population Genetics CMPG lab Bern Switzerland unpublished data). DNA was isolated from front legs using the DNeasy blood and tissue kit (Qiagen). PCR reactions were carried out as described in Gaublomme et al. (2003) and Keller & Largiadèr (2002), and PCR products were resolved on an ABI3130 automated DNA sequencer (Applied Biosystems) using an internal size standard (GS500Liz, Applied Biosystems). Microsatellite allele sizing and binning were conducted with GENEMAPPER v3.7 (Applied Biosystems).

Table 1 - Information on forest fragments and measures of genetic diversity at seven microsatellite loci in 29 *Carabus problematicus* populations. (K) indicates that these forests are part of the former Kolenwoud, N is the number of individuals per population, measures of genetic diversity were mean number of alleles per locus (A), average observed heterozygosity (H_o), average expected heterozygosity (H_e) and allelic richness based on 26 individuals (AR).

Forest fragment	Population	Forest size	N	A	H_o	H_e	AR
Brakel	Brak	243	44	4.429	0.490	0.510	28.86
Inslag	Brasin	394	38	4.429	0.436	0.425	28.29
Buggenhout	Bugg	176	61	3.714	0.345	0.373	22.65
Buzenol	Buze	16200	41	4.857	0.501	0.587	33.26
Chanly	Chanly	46199	40	5.714	0.589	0.669	38.28
Fernelmont	Fernelm	168	29	3.286	0.443	0.498	22.92
Gasthuisbos (K)	Gasth	39	33	3.714	0.486	0.483	25.49
Harentbeek	Har	241	38	4.857	0.500	0.525	31.59
Moelenbusch	HVMuhl	25000	39	5.143	0.530	0.542	32.92
Ruhrbusch	HVRuhr	15310	39	5.000	0.530	0.565	33.09
Withoefse Heide	Kalm	70	38	4.143	0.426	0.453	26.50
Kluisbos	Kluis	274	39	4.286	0.402	0.465	28.45
Bruinbos	Make	82	54	4.143	0.460	0.507	25.59
Meerdaalwoud (K)	Meerddr	1575	36	5.714	0.569	0.578	37.57
Meerdaalwoud (K)	MeerJ+60	1575	40	6.286	0.543	0.564	39.23
Meerdaalwoud (K)	MeerJ-40	1575	40	5.714	0.569	0.575	36.49
Meerdaalwoud (K)	MeerJ-60	1575	40	6.143	0.546	0.568	39.38
Meerdaalwoud (K)	Meerdkl	1575	35	5.571	0.559	0.567	36.54
Meerdaalwoud (K)	MeerO+60	1575	41	5.429	0.495	0.557	35.34
Meerdaalwoud (K)	MeerO-115	1575	37	5.857	0.482	0.515	37.31
Amerloo-Asbroek	Ppame	152	39	4.429	0.367	0.470	29.28
Peerdsbos	Ppeer	280	31	4.000	0.417	0.436	27.21
Rodebos (K)	Rode	104	38	5.286	0.415	0.470	34.28
Rodebos (K)	Roval	104	36	5.286	0.450	0.473	34.99
Soree	Soree?	30	48	4.571	0.492	0.517	29.40
Veursbos	Voer15	1031	37	4.143	0.467	0.472	27.99
Alserbos	Voer7	77	41	3.286	0.380	0.408	22.20
Soignes (K)	ZONP	5103	37	5.571	0.530	0.583	36.88
Soignes (K)	ZONRK	5103	36	5.143	0.572	0.584	34.27

Statistical analyses

We first tested for deviations from Hardy-Weinberg-equilibrium (HWE) and linkage disequilibrium using a Markov chain method implemented in GENEPOP 3.4 (Raymond & Rousset 1995, Rousset 2008) for each locus and for each population (n=29). Deviations from Hardy-Weinberg proportions were quantified by the unbiased estimator of Wright's inbreeding coefficient F_{IS} according to Weir & Cockerham (1984). Significance levels were adjusted for multiple testing using sequential Bonferroni corrections (Rice 1989). Overall F_{ST} was calculated in FSTAT ver 2.9.3 (Goudet 2001).

For each population (n=29), genetic diversity was assessed as average expected heterozygosity (H_e), average observed heterozygosity (H_o) and average number of alleles per locus (A) using POP100GENE 1.1.02.

(http://www.montpellier.inra.fr/URLB/pop100gene/pop100gene.html) and allelic richness (AR; El Mousadik & Petit 1996) as a sample size independent measure of genetic diversity in FSTAT ver 2.9.3 (Goudet 2001). In order to test whether genetic diversity decreased with decreasing patch size, the diversity indices averaged over each forest fragment (n=21) were correlated (Spearman rank order correlation coefficient) to log transformed forest area in STATISTICA 6.0 (StatSoft Inc. 1998). In order to visualize the genetic variation across the landscape, we performed a PCA analysis and estimated the percent of inertia of each PCA axis using GENALEX version 6.41 (Peakall & Smouse 2006).

To identify putative recent reduction in effective population size, we used the heterozygosity excess test implemented in BOTTLENECK 1.2.02 (Piry et al. 1999) for each population (n=29). This software simulates the coalescence process for loci at mutation-drift equilibrium, evolving under a two-phase model of mutation (TPM) as recommended for microsatellites (Cornuet & Luikart 1996) with 1000 iterations for significance testing using Wilcoxon signed rank tests.

Geographic distances between the forest plots were obtained using ARCVIEW version 3.1 (ESRI). The forest layer was based on CORINE land cover 2006 seamless vector data version 13 (02/2010; European Environment Agency 2010) using land cover codes 141 (green urban areas), 311 (broad-leaved forest), 312 (coniferous forest) and 313 (mixed forest), the Flemish Forest Inventory (Waterinckx & Roelandt 2001) and own digitization for the forest fragments Brugmanpark and Verrewinkelbos. An isolation-by-distance model (Slatkin 1993) was used to assess the correlation between genetic and geographic distances (n=21). One central plot per fragment was selected to perform the analyses. Significance was evaluated in GENEPOP with a Mantel test (Mantel, 1976) with 10000 permutations (Raymond & Rousset 1995, Rousset 2008) using (F_{ST}/1-F_{ST}) against the natural logarithm of geographic distance. We also applied a second isolation-by-distance analysis to the spatially more restricted set of populations originating from Kolenwoud remnants near Brussels. To investigate the relative effects of genetic drift and gene flow among patches in more detail, we compared the isolation-by-distance pattern within fragments of the former Kolenwoud with the pattern observed among these forest fragments (Fig 2). For this restricted set of populations, we also quantified the partitioning of genetic variation at different hierarchical levels i.e. among forests (n=4); among populations within forests (n=12) and among individuals within populations by means of AMOVA (Excoffier et al. 1992) as implemented in ARLEQUIN 2.0 (Schneider et al. 2000).

To assess the relative importance of forest size and geographical isolation on the genetic structure, we used a hierarchical Bayesian linear model implemented in GESTE v.2.0 (Foll & Gaggiotti 2006). Here, population specific F_{ST}'s are estimated (n=29) and related to a set of explanatory variables in a linear model. The estimation of population specific F_{ST}'s is based on the degree of differentiation between the estimated allele frequencies of each subpopulation with the estimated allele frequencies of the estimated ancestral population. The program allows to enter a set of nested models that can be compared based on their posterior model probabilities. We thus related F_{ST} values to three explanatory factors i.e. latitudinal distance (Lambert x-coordinate), longitudinal distance (Lambert y-coordinate) and forest area as well as their two- and three-way interactions. Model selection was conducted by means of Bayes Factors. Analyses were run for 300.000 generations, of which the first 50.000 were treated as burn-in period and discarded for parameter estimation.

6.4 Results

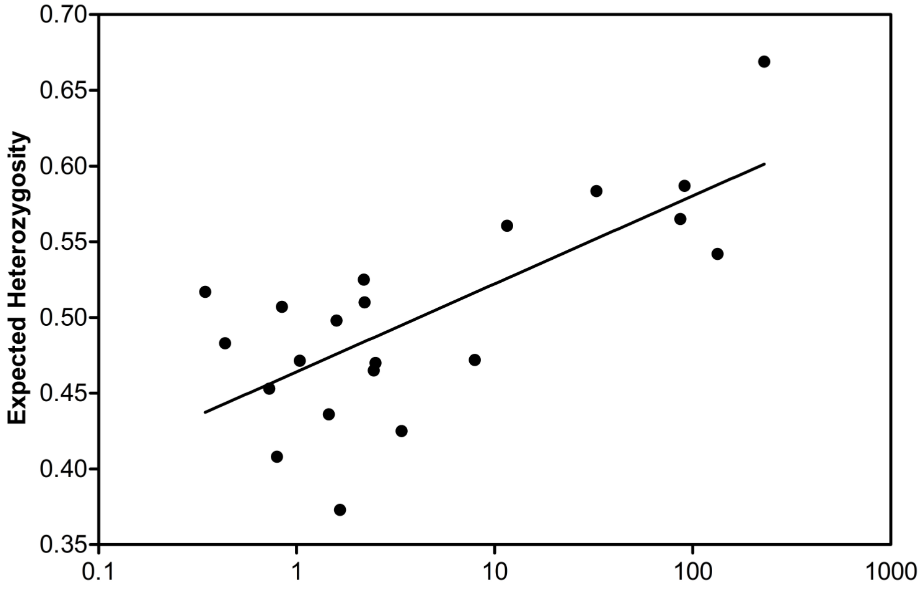
Genetic diversity

The total number of alleles per locus ranged from 7 to 27. Eleven alleles were present at all sampling sites, whereas 21 (17%) alleles were private and only found at ten different localities (Appendix 4). The mean number of alleles (A), expected average heterozygosity (H_e), observed average heterozygosity (H_o) and allelic richness based on 26 individuals (AR) across populations (n=29) averaged to 4.83 (±0.17SE), 0.515 (±0.01SE),0.482 (±0.01SE) and 31.92 (±5.44SE) respectively (Table 1). Locus P99 showed deviations from Hardy-Weinberg-Equilibrium and was withdrawn from further analyses. None of the loci were in linkage disequilibrium in any of the populations.

BOTTLENECK analysis revealed no excess of heterozygosity in any of the populations (n=29) thus, there were no traces of recent genetic bottlenecks. Inbreeding coefficients (F_{IS}) were not significantly different from zero in all populations and for all loci.

Forest area was significantly and positively correlated with expected heterozygosity (n=21, r=0.574, P=0.007, Fig 3A), observed heterozygosity (n=21, r=0.60, P=0.007), mean number of alleles (n=21, r=0.633, P=0.0021, Fig 3B) and allelic richness (n=21, r=0.704, P<0.001), indicating that populations from larger forest fragments were genetically more diverse.

(A)



(B)

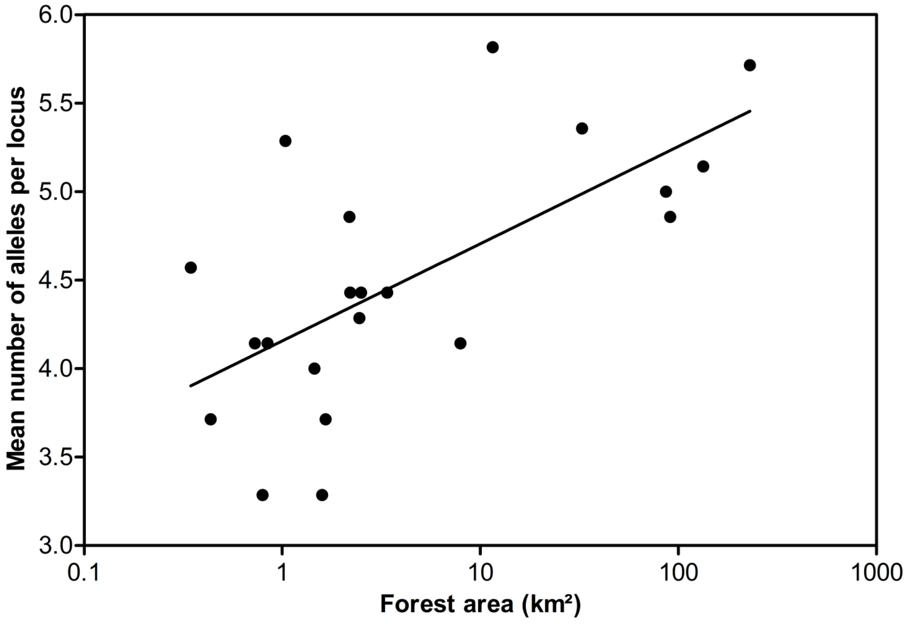


Fig. 3 - Relationship between forest area and (A) average expected heterozygosity and (B) mean number of alleles per locus per population in *Carabus problematicus*.

The results of the PCA are given in Fig 4, wich represents the plane defined by the first two axes of the analyses. The cumulative percentage explained by the two first principal axes was 60.96%. A clear separation can be seen for the Campine region, Voer region, High Fens and Buggenhout.

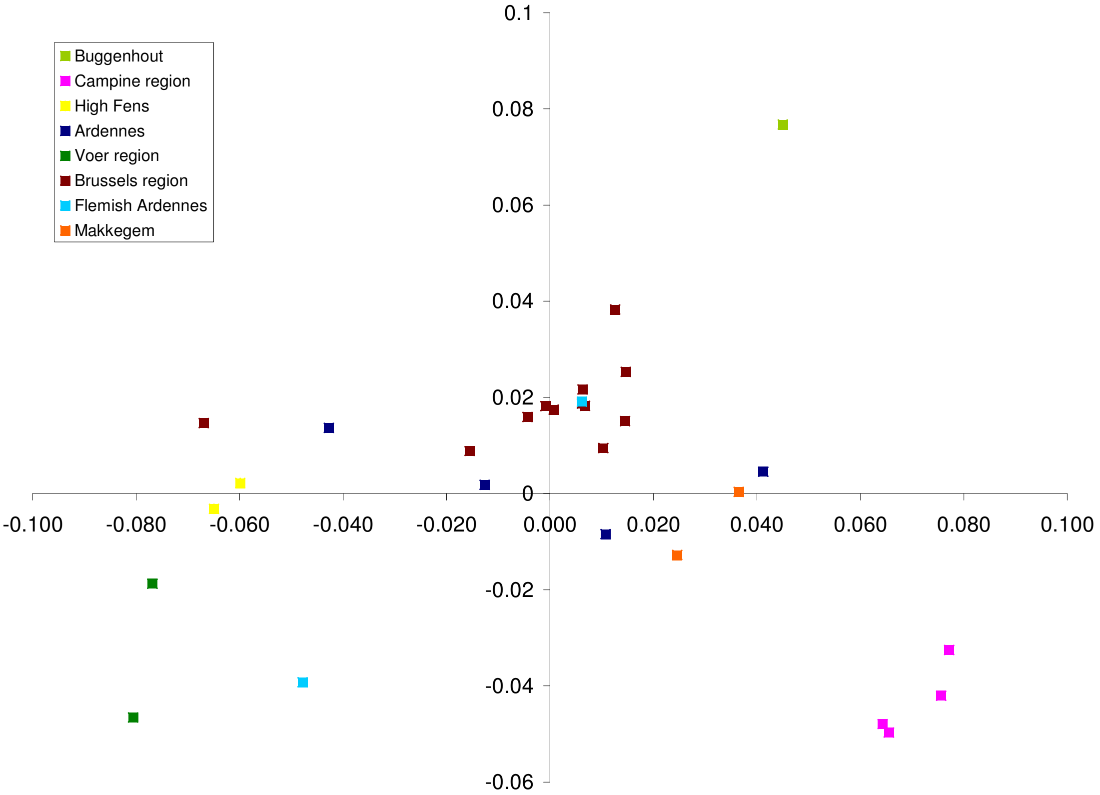


Fig. 4 – Principal component analysis based on allele frequencies of 29 populations.

Genetic differentiation in response to geographic distance and forest area

Overall differentiation among populations (n=29) was relatively high with $F_{ST} = 0.12$ ($P \leq 0.001$). Mantel tests showed a significant correlation between genetic distances and geographic distances when considering all forest fragments (n=21, r=0.440, P=0.005; Fig 5A). This pattern emerged primarily by the small genetic distance of some very close forests. When restricting our analysis to the Kolenwoud fragments, no correlation between geographic and genetic distance was found (n=4, r=0.429, P=0.147; filled circles in Fig 5B). Likewise, when the relationship between geographic and genetic distance was investigated among populations within fragments, no significant correlation was observed (r=0.330, P=0.987; open circles in Fig 5B). This was also shown by AMOVA, where the variance among the four different forest fragments of the Kolenwoud (6.51%; P= 0.004) was higher compared to the variance among populations within forest fragments (0.24%; P= 0.05). Most of the variation however was explained by the variance within populations (93.39%, p<0.001).

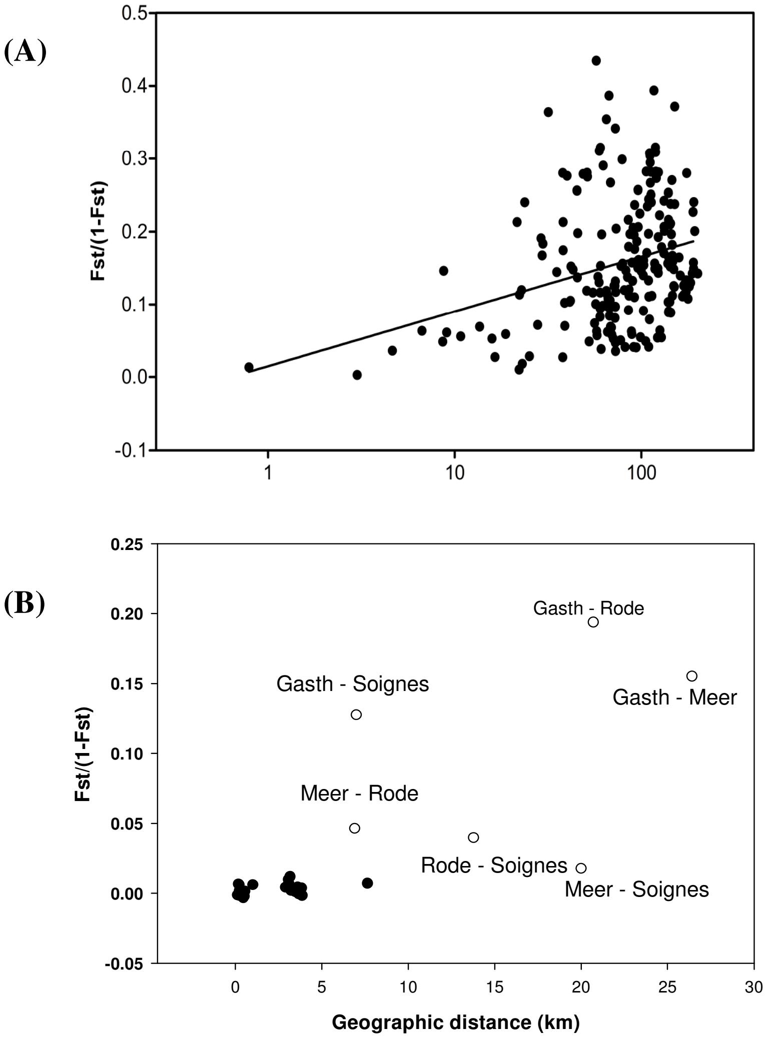


Fig. 5 – (A) Relationship between geographic (km) and genetic (F_{ST} /(1- F_{ST})) distance for all sampled forest fragment populations of *Carabus problematicus* and (B) for Kolenwoud populations only. Open circles represent comparisons of populations from the same forest fragment filled circles represent population comparisons from different forest fragments.

The linear model approach in GESTE (Foll & Gaggiotti 2006) did not support a model that included the geographic positioning of fragments. Highest support was found for a simple regression model that only included the effect of forest area (posterior probability = 0.837). Forest area had a negative effect on population specific F_{ST}-values, indicating that genetic differentiation decreased with increasing forest size (Fig 6). Hence, populations from smaller forest fragments were significantly more differentiated than those from larger forest fragments.

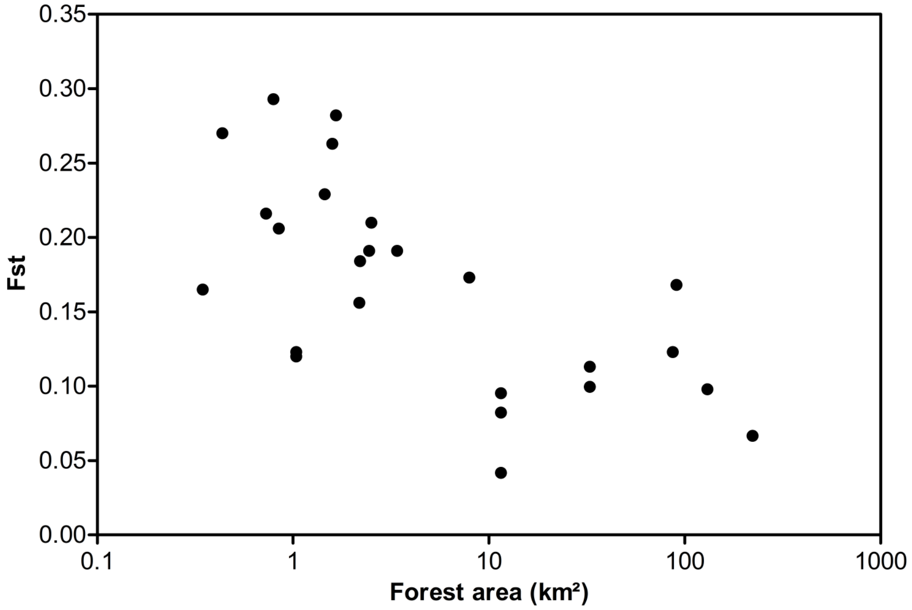


Fig. 6 - Relationship between population specific F_{ST}-values and forest area in *Carabus problematicus*.

6.5 Discussion

This population genetic study on the forest specialist and wingless beetle *C. problematicus* shows that populations from different forest fragments are, in general, genetically differentiated. The mean observed F_{ST} value (F_{ST}=0.12) is rather high as compared to other ground beetle studies based on microsatellites, where overall F_{ST} values ranged between 0.008 and 0.082 for five different ground beetles (Brouat et al. 2003, Dhuyvetter et al. 2005, Drees et al. 2008, Lagisz et al. 2010). Only Garnier et al. (2004) reported a much higher value (F_{ST} = 0.335) for *C. solieri* Dejean 1826. The authors explained this result with the limited dispersal capacity of this species and its particular habitat surrounded by a heterogeneous matrix with many barriers to gene flow. Though direct comparisons of F_{ST} values of different studies can be misleading since the scale of the studies differ, our study was based on a larger geographic scale (entire Belgium) as compared to the other microsatellite studies mentioned above.

Our linear model approach revealed that forest size is the main factor influencing the genetic structure of *C. problematicus*, wherein smaller forest fragments exhibited a considerably higher genetic differentiation compared to larger forest fragments. This is in strong accordance with population genetic theory wherein the intensity of genetic drift in a population is inversely related to its effective size (Hedrick 2005, Taylor 2003). This demonstrates that at least for our smaller fragments drift processes are only, counteracted by migration among fragments to a minor extent. In case of strong genetic drift, it is expected that particular alleles may become locally extinct, thus eroding genetic diversity (Slatkin 1987, Hanski & Gilpin 1991). That this process is currently ongoing in our study system was evidenced by a positive relationship between all measures of genetic diversity and forest fragment area. A comparable finding was made by Desender (2005) and Drees et al. (2011), who also showed that habitat size influences genetic diversity of ground beetles, albeit in other habitat types. A reduction in allelic diversity can lead to decreased heterozygosity and inbreeding depression, which enhances the risk of local extinction (Saccheri et al. 1998, Frankham et al. 2002). In Belgium, *C. problematicus* was never recorded in forests smaller than 30 hectares.

Although drift processes play a major role in determining the genetic structure, at least in smaller populations, it is challenging to quantify and infer the amount of gene flow and migration among and within fragments. At the larger geographic scale of Belgium, a genetic structure in accordance with an isolation-by-distance model at first suggested equilibrium between drift and gene flow (Hutchison & Templeton 1999). However, a more detailed inspection (Fig 3A) showed that the relationship was primarily caused by the small genetic distance of a few neighboring populations, pointing towards migration events being restricted to those fragments. Yet, it should be noted that a smaller genetic distance among nearby populations remains hard to distinguish from a shared evolutionary history experienced by neighboring populations. The importance to distinguish between current and historical gene flow was also stated by Desender et al. (1999) who demonstrated for *C. problematicus* populations in forests that persisted since the Roman period that isolation-by-distance patterns reflect historical rather than contemporary gene flow. Moreover, isolation by distance patterns could also be confounded if there is spatial autocorrelation in fragment area. However, only a weak and not significant degree of spatial autocorrelation in fragment area was observed (Moran's I = 0.07; p = 0.16).

To account for possible confounding relationships, we conducted a spatially more restricted analysis of the Kolenwoud populations, for which a shared evolutionary history of the sampled populations can be assumed. On this smaller geographic scale, distance among sampled populations did not significantly explain patterns of genetic differentiation neither within as well as among fragments. However, differentiation among populations originating from different fragments was significant and considerably higher compared to genetic differentiation of populations originating from the same fragment, which was almost absent. Visual inspection of the isolation-by-distance relationship between both types of pairwise population comparisons (Fig 3B) showed that this was unlikely to be caused by the larger geographic distance of populations from different fragments. Rather, this analysis suggested that migration, and hence gene-flow, was clearly hampered by the matrix surrounding the fragments rather than by mere geographical distance. This leaves major drift effects as the main factor determining genetic differentiation among populations, and high levels of migration mainly causing among populations within forest fragments (Hutchison & Templeton 1999).

The restricted dispersal capacities of *C. problematicus* (Neuman 1971, Rijsdorp 1980) are most probably not simply due to its absence of flight capability, but also largely based on its stringent habitat requirements. Although this beetle is able to run quite far in a single night (70-75 meters), the matrix between forest fragments is unlikely to be suitable for dispersal. Indeed, in a former study conducted in the same area of Brussels, strict forest specialists were never found outside of forest fragments and even avoided edge habitats (Gaublomme et al. 2008). *C. problematicus* is restricted to large, dark, cool forests without human interference, undisturbed soil and abundant dead wood (Niemelä 1997, Desender et al. 1999). This specialist behavior should reinforce isolation effects if the matrix between two forest fragments is unsuitable for dispersal. Brouat et al. (2003) also showed that non-forested areas were partial barriers to gene flow for the forest specialist species *C. punctatoauratus* and the forest species *C. nemoralis*.

Worldwide, endangered species exist in remnant populations within fragmented landscapes. Due to barriers to dispersal, effective distances between populations increase, and, consequently, gene flow between remaining populations is likely to be strongly reduced or even absent (Biedrzycka & Konopinski 2008). Low colonization ability and low tolerance to environmental changes explain the decline of many forest species (Hanski 2005). As also shown in our study, species with low dispersal abilities may suffer from isolation and drift even at very small geographic distances (Krauss et al. 2003). Even small stretches of highly developed infrastructure and intensive agricultural land might put such forest species at risk.

Results of this population genetic study are also consistent with previous community level studies where carabid assemblages of smaller fragments were found to be significantly less diverse in number of short winged species (Hendrickx et al. 2009). In accordance with these results, stochastic effects most probably play an important role for brachypterous species in smaller fragments and may ultimately drive local populations towards extinction.

Insights into the genetic structure of threatened populations, particularly those existing in fragmented habitats is relevant for the management and conservation. Our study suggests that an unsuitable matrix poses a barrier to species with low dispersal capacity and high habitat requirements. Establishing new forests could therefore be of only limited effect for specialist species, as these patches might be readily colonized. A more suitable management option is to preserve and enlarge existing forest fragments. Corridors connecting remaining habitat fragments would only be useful if they were very large and contain core habitat.

Conclusions

Combined with small population sizes, *C. problematicus* showed low levels of neutral genetic diversity and high levels of population structure in Belgian forest fragments. Our data indicated that the genetic structure was mainly determined by forest size. Given that the geographic location of the patches was far less important, very limited gene flow between populations of remaining forest fragments, caused strong drift in small forest fragments. Fragment area was by far the most important factor explaining genetic structure. Hence, conservation management should aim at keeping forest fragments as large as possible to counteract genetic impoverishment.

6.6 Acknowledgements

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6.8 Appendix

Appendix 4 – Allele frequencies from seven microsatellite loci in 29 populations.

Locus	allele	brak	brasin	bugg	buze	chanly	fernelmont	gast	harent	hvmuhl	hvruhr	kalm	kluis	make	meerdr	meerJ+60	meerJ-40	meerJ-60	meerkl	meerO+60	meer0-115	peer	amerloo	rode	rov	soree	voer15	voer7	zonP	zonRK
P44																														
Gene Number		88	76	122	82	80	58	66	76	78	78	76	78	108	72	80	80	80	70	82	74	78	62	76	72	96	74	82	74	72
Allele Number		2	3	2	2	4	2	2	3	3	2	5	3	2	2	2	3	2	4	3	2	5	1	2	3	3	2	1	2	3
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.09	0.11	0.01	0.10	0.20	0.05	0.17	0.05	0.10	0.22	0.03	0.06	0.07	0.10	0.10	0.15	0.13	0.14	0.11	0.08	0.01	0.00	0.03	0.01	0.06	0.08	0.00	0.23	0.17
	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
	6	0.91	0.87	0.99	0.90	0.69	0.95	0.83	0.92	0.89	0.78	0.91	0.91	0.93	0.90	0.90	0.83	0.88	0.83	0.87	0.92	0.92	1.00	0.97	0.96	0.92	0.92	1.00	0.77	0.82
	7	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.03	0.03	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
	8	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Heterozygote Proportion		0.14	0.16	0.02	0.15	0.20	0.10	0.33	0.11	0.18	0.13	0.13	0.13	0.15	0.19	0.15	0.30	0.15	0.14	0.17	0.11	0.08	0.00	0.05	0.03	0.13	0.11	0.00	0.41	0.31
Gene Diversity		0.17	0.24	0.02	0.18	0.49	0.10	0.28	0.15	0.21	0.35	0.18	0.17	0.14	0.18	0.18	0.30	0.22	0.30	0.24	0.15	0.15	0.00	0.05	0.08	0.16	0.15	0.00	0.36	0.31
P40																														
Gene Number		94	80	126	80	80	58	74	78	78	80	78	80	108	72	80	80	78	72	82	76	68	58	74	66	96	76	80	76	68
Allele Number		2	3	3	3	3	2	4	2	4	4	2	2	3	3	3	3	6	3	3	3	2	2	3	3	3	3	4	3	3
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
	2	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.03	0.16	0.29	0.00	0.15	0.00	0.28	0.38	0.00	0.00	0.00	0.01	0.04	0.04	0.08	0.03	0.02	0.04	0.00	0.00	0.04	0.02	0.10	0.09	0.09	0.01	0.03
	4	0.53	0.30	0.10	0.35	0.35	0.19	0.81	0.33	0.53	0.54	0.55	0.96	0.42	0.47	0.39	0.38	0.37	0.42	0.31	0.49	0.54	0.50	0.39	0.38	0.55	0.80	0.85	0.40	0.50
	5	0.47	0.69	0.87	0.49	0.36	0.81	0.03	0.67	0.18	0.03	0.45	0.04	0.57	0.51	0.58	0.59	0.47	0.56	0.67	0.47	0.46	0.50	0.57	0.61	0.34	0.11	0.05	0.59	0.47
	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heterozygote Proportion		0.51	0.53	0.18	0.63	0.50	0.38	0.35	0.31	0.56	0.48	0.44	0.08	0.44	0.50	0.40	0.48	0.49	0.61	0.34	0.50	0.38	0.38	0.43	0.55	0.60	0.29	0.20	0.47	0.56
Gene Diversity		0.50	0.44	0.23	0.62	0.67	0.31	0.32	0.45	0.62	0.57	0.50	0.07	0.50	0.52	0.52	0.52	0.64	0.52	0.46	0.54	0.50	0.51	0.53	0.50	0.57	0.34	0.27	0.50	0.54
P97																														
Gene Number		80	76	128	56	80	58	72	82	78	80	80	72	108	70	80	80	80	72	82	78	76	52	72	64	96	76	82	58	58
Allele Number		4	5	3	3	5	2	3	3	4	4	4	5	3	6	4	4	5	6	4	5	4	5	5	4	3	3	3	4	5
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.01	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.28	0.36	0.12	0.54	0.25	0.47	0.08	0.15	0.14	0.09	0.54	0.29	0.10	0.14	0.03	0.09	0.09	0.01	0.10	0.04	0.15	0.27	0.10	0.06	0.19	0.04	0.26	0.14	0.16
	8	0.64	0.17	0.62	0.43	0.61	0.53	0.88	0.45	0.83	0.84	0.19	0.57	0.44	0.56	0.69	0.65	0.69	0.65	0.62	0.67	0.16	0.23	0.74	0.86	0.80	0.84	0.63	0.69	0.64
	9	0.08	0.03	0.27	0.04	0.03	0.00	0.00	0.00	0.00	0.05	0.08	0.08	0.00	0.01	0.00	0.04	0.04	0.01	0.02	0.03	0.05	0.08	0.04	0.05	0.00	0.12	0.11	0.07	0.07
	10	0.00	0.43	0.00	0.00	0.00	0.00	0.04	0.40	0.00	0.00	0.20	0.04	0.45	0.24	0.28	0.23	0.18	0.28	0.26	0.26	0.65	0.40	0.11	0.03	0.01	0.00	0.00	0.10	0.12
	11	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02

Heterozygote Proportion		0.50	0.76	0.53	0.46	0.53	0.45	0.22	0.61	0.21	0.33	0.73	0.39	0.50	0.54	0.53	0.63	0.45	0.39	0.44	0.49	0.66	0.69	0.44	0.28	0.27	0.26	0.59	0.45	0.55
Gene Diversity		0.52	0.66	0.54	0.54	0.56	0.51	0.23	0.62	0.29	0.29	0.64	0.59	0.59	0.62	0.46	0.52	0.49	0.50	0.54	0.49	0.54	0.72	0.44	0.26	0.33	0.28	0.53	0.50	0.56
P98																														
Gene Number		88	70	128	70	80	58	74	80	78	80	80	80	104	78	80	80	80	62	82	78	78	54	76	64	96	76	82	66	70
Allele Number		7	6	7	9	11	4	5	6	12	11	6	7	6	12	13	12	10	10	11	10	7	6	8	10	12	9	6	11	9
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	2	0.17	0.07	0.00	0.04	0.15	0.12	0.00	0.03	0.10	0.09	0.06	0.06	0.02	0.09	0.03	0.06	0.03	0.08	0.02	0.05	0.09	0.04	0.08	0.06	0.06	0.01	0.24	0.18	0.36
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.00	0.00	0.10	0.06	0.00	0.15	0.00	0.04	0.01	0.00	0.03	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.04	0.00	0.00	0.12	0.13
	5	0.43	0.01	0.31	0.34	0.21	0.07	0.00	0.00	0.03	0.00	0.00	0.46	0.00	0.14	0.26	0.24	0.23	0.21	0.29	0.18	0.03	0.02	0.07	0.20	0.22	0.03	0.05	0.05	0.13
	6	0.27	0.86	0.30	0.26	0.33	0.55	0.14	0.45	0.19	0.31	0.78	0.33	0.52	0.28	0.38	0.35	0.38	0.27	0.32	0.49	0.62	0.82	0.66	0.48	0.15	0.30	0.28	0.41	0.29
	7	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01
	8	0.02	0.00	0.00	0.13	0.05	0.26	0.00	0.00	0.40	0.24	0.00	0.06	0.00	0.08	0.04	0.05	0.05	0.11	0.06	0.05	0.06	0.00	0.03	0.02	0.26	0.28	0.34	0.02	0.01
	9	0.00	0.00	0.00	0.00	0.01	0.00	0.34	0.00	0.00	0.01	0.00	0.00	0.00	0.05	0.01	0.05	0.00	0.03	0.02	0.00	0.00	0.00	0.01	0.03	0.00	0.05	0.00	0.02	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.03	0.04	0.05	0.00	0.02	0.04	0.00	0.00	0.03	0.06	0.00	0.00	0.00	0.00	0.03
	11	0.00	0.01	0.02	0.03	0.01	0.00	0.00	0.01	0.01	0.05	0.01	0.00	0.01	0.05	0.01	0.00	0.00	0.02	0.01	0.00	0.09	0.02	0.05	0.05	0.02	0.03	0.00	0.08	0.00
	12	0.06	0.03	0.00	0.00	0.05	0.00	0.18	0.43	0.08	0.14	0.01	0.04	0.42	0.12	0.08	0.11	0.16	0.13	0.13	0.06	0.00	0.00	0.08	0.06	0.04	0.16	0.07	0.06	0.03
	13	0.03	0.01	0.18	0.04	0.06	0.00	0.20	0.00	0.04	0.06	0.13	0.03	0.01	0.13	0.08	0.05	0.05	0.07	0.09	0.08	0.10	0.09	0.00	0.02	0.01	0.12	0.01	0.05	0.00
	14	0.01	0.00	0.07	0.00	0.05	0.00	0.00	0.00	0.08	0.04	0.01	0.00	0.00	0.03	0.03	0.01	0.01	0.07	0.00	0.03	0.00	0.02	0.00	0.02	0.01	0.03	0.00	0.02	0.01
	15	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00
	16	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.04	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	17	0.00	0.00	0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00
	18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heterozygote Proportion		0.68	0.23	0.70	0.69	0.85	0.69	0.76	0.68	0.74	0.78	0.45	0.73	0.60	0.85	0.73	0.75	0.85	0.94	0.71	0.69	0.39	0.37	0.53	0.78	0.79	0.87	0.68	0.64	0.74
Gene Diversity		0.71	0.26	0.77	0.80	0.82	0.62	0.78	0.62	0.79	0.82	0.38	0.68	0.56	0.86	0.78	0.81	0.78	0.85	0.79	0.72	0.60	0.33	0.55	0.72	0.85	0.80	0.75	0.78	0.77
P55																														
Gene Number		72	70	126	62	80	58	74	76	76	76	78	74	102	68	80	78	78	70	82	74	60	52	68	64	96	76	82	52	64
Allele Number		2	2	2	4	3	3	2	4	2	4	2	3	4	3	5	3	3	4	2	3	3	3	3	4	2	2	2	4	3
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	2	0.88	0.93	0.97	0.66	0.63	0.64	0.66	0.76	0.55	0.54	0.92	0.65	0.71	0.78	0.61	0.73	0.77	0.79	0.76	0.81	0.88	0.89	0.74	0.81	0.66	0.49	0.49	0.64	0.63
	3	0.13	0.07	0.03	0.19	0.19	0.17	0.34	0.09	0.45	0.41	0.08	0.34	0.18	0.16	0.34	0.23	0.21	0.17	0.24	0.18	0.03	0.02	0.15	0.09	0.34	0.51	0.51	0.29	0.27
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.00	0.00	0.00	0.10	0.19	0.19	0.00	0.13	0.00	0.04	0.00	0.01	0.09	0.06	0.03	0.04	0.03	0.01	0.00	0.01	0.08	0.10	0.12	0.06	0.00	0.00	0.00	0.06	0.11
	7	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
Heterozygote Proportion		0.19	0.14	0.03	0.42	0.55	0.31	0.51	0.40	0.63	0.66	0.10	0.32	0.37	0.35	0.43	0.44	0.41	0.29	0.34	0.30	0.10	0.23	0.32	0.19	0.48	0.50	0.39	0.31	0.41
Gene Diversity		0.22	0.14	0.06	0.52	0.55	0.54	0.45	0.40	0.50	0.55	0.14	0.47	0.47	0.37	0.52	0.42	0.37	0.36	0.37	0.32	0.22	0.21	0.43	0.33	0.46	0.51	0.51	0.52	0.54
P104481																														
Gene Number		88	80	128	54	80	54	72	70	78	80	80	74	106	56	80	80	76	66	82	80	58	60	78	68	94	74	82	66	66
Allele Number		4	3	4	5	5	4	4	3	3	3	3	3	3	3	3	4	4	3	4	3	3	3	3	3	2	2	2	3	4
	1	0.24	0.14	0.45	0.28	0.31	0.32	0.25	0.26	0.45	0.30	0.35	0.28	0.25	0.43	0.35	0.48	0.33	0.30	0.37	0.40	0.31	0.28	0.18	0.28	0.33	0.31	0.13	0.30	0.27
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.22	0.00	0.01	0.07	0.18	0.28	0.08	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.01	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.51	0.70	0.53	0.24	0.35	0.11	0.32	0.60	0.50	0.60	0.30	0.62	0.65	0.43	0.50	0.41	0.53	0.56	0.51	0.55	0.45	0.45	0.69	0.50	0.67	0.69	0.87	0.59	0.62
	7	0.03	0.16	0.01	0.20	0.11	0.30	0.35	0.14	0.05	0.10	0.35	0.00	0.10	0.14	0.15	0.10	0.12	0.14	0.11	0.05	0.24	0.27	0.13	0.22	0.00	0.00	0.00	0.11	0.09
	8	0.00	0.00	0.00	0.20	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heterozygote Proportion		0.66	0.53	0.61	0.67	0.70	0.74	0.69	0.54	0.56	0.63	0.50	0.60	0.43	0.68	0.65	0.53	0.55	0.64	0.51	0.53	0.48	0.67	0.39	0.56	0.40	0.46	0.22	0.58	0.58
Gene Diversity		0.64	0.47	0.52	0.79	0.74	0.74	0.72	0.56	0.55	0.55	0.67	0.53	0.51	0.62	0.61	0.60	0.61	0.58	0.60	0.54	0.66	0.66	0.48	0.63	0.45	0.43	0.24	0.56	0.54
P105136																														
Gene Number		88	76	126	72	78	56	72	74	78	80	72	76	110	74	78	80	74	68	82	76	82	62	78	68	96	72	82	72	74
Allele Number		10	9	5	8	9	6	6	13	8	7	7	7	8	11	14	11	13	9	11	15	7	8	13	10	7	8	5	12	9
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.06	0.01	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.05	0.01	0.00	0.03	0.12	0.00	0.22	0.03	0.00	0.00	0.00	0.00	0.04	0.04	0.01	0.01	0.01	0.02	0.02	0.04	0.00	0.00	0.03	0.04	0.17	0.00	0.00	0.11	0.11
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.32	0.04	0.70	0.08	0.14	0.04	0.60	0.05	0.18	0.16	0.01	0.18	0.03	0.19	0.24	0.18	0.22	0.24	0.18	0.34	0.05	0.00	0.33	0.38	0.24	0.17	0.06	0.25	0.30
	5	0.01	0.00	0.00	0.01	0.09	0.00	0.00	0.16	0.13	0.18	0.00	0.00	0.26	0.11	0.10	0.15	0.08	0.10	0.10	0.07	0.00	0.00	0.13	0.03	0.15	0.11	0.17	0.03	0.00
	6	0.26	0.36	0.10	0.08	0.15	0.14	0.00	0.12	0.17	0.04	0.54	0.30	0.15	0.14	0.19	0.16	0.19	0.18	0.16	0.07	0.57	0.57	0.08	0.12	0.00	0.13	0.00	0.19	0.12
	7	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00

8	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.01	0.00	0.00	0.00	0.06	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.03	0.00	0.00	0.00
10	0.00	0.00	0.00	0.13	0.05	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
11	0.02	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.02	0.00	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.01	0.00
12	0.03	0.28	0.17	0.56	0.27	0.52	0.00	0.20	0.09	0.19	0.19	0.37	0.23	0.04	0.09	0.08	0.08	0.10	0.16	0.08	0.20	0.23	0.26	0.21	0.26	0.13	0.11	0.11	0.12
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
14	0.00	0.20	0.00	0.06	0.14	0.07	0.04	0.19	0.30	0.25	0.10	0.03	0.29	0.20	0.12	0.20	0.19	0.21	0.12	0.17	0.07	0.03	0.03	0.10	0.13	0.38	0.62	0.08	0.15
15	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.04	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.01	0.01	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.09	0.05	0.00	0.00	0.00	0.21	0.06	0.01	0.00	0.00	0.13	0.00	0.01	0.08	0.03	0.04	0.05	0.06	0.07	0.05	0.05	0.11	0.01	0.00	0.00	0.00	0.00	0.06	0.05
21	0.09	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.09	0.16	0.00	0.08	0.00	0.11	0.08	0.15	0.11	0.07	0.07	0.08	0.04	0.02	0.01	0.02	0.00	0.06	0.04	0.08	0.10
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.03	0.01	0.00	0.01	0.03	0.05	0.00	0.01	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.03	0.07	0.00	0.00	0.00	0.00	0.00
26	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heterozygote Proportion	0.75	0.71	0.35	0.50	0.80	0.43	0.53	0.87	0.82	0.73	0.64	0.58	0.73	0.87	0.92	0.88	0.92	0.91	0.95	0.76	0.49	0.58	0.74	0.77	0.77	0.78	0.59	0.86	0.87
Gene Diversity	0.81	0.76	0.48	0.66	0.85	0.67	0.59	0.88	0.83	0.83	0.65	0.74	0.78	0.88	0.87	0.86	0.86	0.85	0.89	0.84	0.63	0.63	0.81	0.79	0.82	0.80	0.57	0.87	0.85

Top: *Carabus problematicus*, bottom: *Carabus violaceus* © Konjev Desender

Chapter 7

IMPACT OF FRAGMENTATION ON THE GENETIC STRUCTURE OF THE FOREST GENERALIST *CARABUS VIOLACEUS,* WITH COMPARISON TO THE FOREST SPECIALIST *CARABUS PROBLEMATICUS* (COLEOPTERA: CARABIDAE)

Chapter 7

Impact of fragmentation on the genetic structure of the forest generalist *Carabus violaceus,* with comparison to the forest specialist *Carabus problematicus (Coleoptera: Carabidae)*

EVA GAUBLOMME, TIM ADRIAENS AND FREDERIK HENDRICKX

7.1 Summary

Ground beetles often persist in highly fragmented forests due to human alterations of the landscape. In particular for species that are restricted in their dispersal capacity, such as wingless ground beetles bound to forest habitats. The extent to which populations may exchange genes among isolated populations remains poorly quantified. We therefore investigated population genetic patterns of the forest generalist *Carabus violaceus* in a fragmented landscape by means of microsatellite allele frequencies in 19 different populations from both a contiguous forest fragment and highly fragmented forest patches in the centre of Belgium.

We observed a lower genetic diversity and a higher differentiation level in the fragments as compared to the plots in the contiguous forest. There was evidence of a reduced mobility and gene flow in the fragments compared to the contiguous forest, including a significant isolation by distance pattern. Furthermore, we compared the results of this forest generalist species to the forest specialist species *C. problematicus*. Differentiation was significantly higher and indicated an elevated level of gene flow for the forest specialist species. Our results suggest a positive association between levels of gene flow and the movement behavior of the two species.

Keywords: microsatellites, isolation by distance, forest fragmentation, gene flow, differentiation, ground beetles, Carabidae.

7.2 Introduction

Fragmentation of natural habitats increased dramatically by human activities and forms a serious threat to many insect species for many reasons (Fahrig 2003, Ewers & Didham 2006). It reduces the suitable area for organisms, leads to the isolation and size reduction of remnant populations, and increases the risk of local extinction (Saccheri et al. 1998). In western Europe, clearance of forests for agriculture made the remaining forest extremely patchy (Honnay et al., 2005). The patches are differing in quality, age, size and isolation and the local populations of species living in the forest fragments either become isolated or may disperse between forest patches, depending on the distance between patches, the properties of the matrix and the dispersal capability of these species (Öckinger & Smith 2008, Prevedello & Vieira 2010).

It is commonly accepted that specialist species suffer more from the consequences of habitat fragmentation compared to generalist species (Didham et al. 1996, Brouat et al. 2003, Krauss et al. 2003, Steffan-Dewenter 2003, Vandergast & Gillespie, 2004). Due to the low potential of specialized species to cross the landscape matrix situated between the different fragments, gene flow is reduced and leads to genetic impoverishment (Saunders et al. 1991, Frankham 1995, Westemeier et al. 1998) of the populations living within the fragments and stronger genetic differentiation (Harrison & Hastings 1996, Hutchinson & Templeton 1999) among them (i.e. drift effects). Moreover, a high level of genetic differentiation also demonstrates that the ability of a population to recolonize vacant patches may be seriously reduced, which may ultimately lead to extinction of populations beyond the local scale.

In a previous study (chapter 6) we investigated the relative effects of patch size, as a proxy of population size, and degree of isolation on the composition of neutral genetic variation of the specialist ground beetle *Carabus problematicus*. Patch size, rather than geographic distance among the populations, clearly appeared the most important factor causing drift effects, suggesting that exchange of individuals between the fragments was virtually absent.

In the central part of Belgium we find a unique setting to study the effects of fragmentation on forest patches within a common historical context. One former large and contiguous population, residing within the historic Kolenwoud forest, became fragmented into several different populations that differ profoundly in population size. The time at which they were separated is well documented on the basis of historical maps and forms an ideal set up to study the population genetic effects of habitat fragmentation on a small geographic scale.

Here, we make a detailed population genetic study of the forest generalist *Carabus violaceus* in this region and compare the degree of genetic structuring among different populations of this large fragment with the degree of differentiation among different forest fragments. More specifically, we attempt to investigate the population genetic consequences of forest area, forest history and degree of geographic isolation within this region.

Moreover, these results are compared with those of the forest specialist species *C. problematicus* that was studied previously within the same context in this region. Given that *C. violaceus* has a highly comparable life history but is generally less restricted in its habitat preference, we hypothesize that it is less prone to the effects of fragmentation compared to *C. problematicus*.

7.3 Material and methods

Study species

C. violaceus is a species commonly found in different types of forests, but preferably in humus-rich and older forests. In contrast to *C. problematicus*, it can be found in small numbers in open landscapes and is therefore expected to be less prone to the effects of fragmentation (Desender et al. 2004). *C. problematicus* is absent from the majority of the studied fragments. Nevertheless data from archaeological sites demonstrate that the species was previously much more common in the central part of Belgium (Desender et al. 1999). Local extinction of populations as a consequence of habitat fragmentation had been put forward as the most likely explanation for its regional decline (Desender et al. 2004). In contrast to *C. problematicus*, small numbers of *C. violaceus* have been found in matrix habitat such as arable land, grassland, hedges, garden (Gaublomme et al. 2008), which is expected to counteract the negative effects of fragmentation to a fairly large extent as it might strongly reduce processes related to genetic drift and enables the species to recolonize fragments that were previously extinct.

The two beetle species are very similar with respect to life history and general morphology and share a similar distribution throughout the Atlantic part of Europe (Turin 2000), *C. problematicus* is also found in Central Europe and Scandinavia. They are large (between 22 mm and 35 mm), short winged and therefore unable to fly (Wachmann et al. 1995). Nevertheless, larger ground beetles of the genus *Carabus* are able to cover distances on the ground of more than 10 m in 24 h and a few hundred meters in longer time-spans (Thiele

1977). According to Blumenthal (1981) 4 km of open grassland would prevent *C. violaceus* from colonizing neighboring forests and they avoid to cross paved roads (Mader 1984, Keller et al. 2004). Both species are nocturnal (Lindroth 1985). The newly emerged adults occur in summer and reproduce mainly in August. Some adults hibernate and enter upon a second breeding in the following summer (Lindroth, 1985).

Study area and sampling design

The Kolenwoud or Silva Carbonaria was a medieval forest, covering the central part of Belgium with a total area of 12000 ha. From the 12th century onwards, it was partly subdivided into smaller fragments and its total area reduced to 10382 ha in the 16th century (Van de Velde, 1992). In the 18th century, the largest part, constituting for its majority the current Soignes forest, had a total area of 10800 ha (Lefèbvre, 1997). From 1843 until now the remaining forest retained its current area of 4383 ha and forms together with other remnants (Table 1) the last remains of the former Kolenwoud. These forests all have the same history and became isolated at different times (Table 1).

Populations of *Carabus violaceus* were collected in forests in and around Brussels (50°50'-50°59'N, 4°11'-4°25'E), Belgium (Fig 1). In total we investigated 19 sampling locations (Table 1), from which eleven are located within the contiguous large Soignes forest (4383 ha). Since we were not able to define populations in this continuous habitat, we selected 11 sampling plots distributed over the forest. Another six sampling locations were selected in the remaining fragments i.e. Haller forest, Meerdaal forest, Ronde forest, Verrewinkel forest, Gasthuis forest and Kleet forest, which range in size from 5.4 ha to 175.2 ha and were once connected to the large Soignes forest, but isolated from this forest at a different time in history due to fragmentation. Two forests (Laarbeek forest and Buggenhout forest) have a different history and it is not known whether they were ever connected to the Soignes forest. All studied forests are ancient and mainly consist of beech except for Buggenhout forest which is a mixture of beech and oak, but was originally also dominated by beech.

At each site, between 60 and 100m from the forest edge, six pitfalls (at about 5m from each other) were installed, without fixative, in order to obtain live beetles. The top (5 cm) of a plastic bottle (diameter = 10cm) was cut off and put inversely in the lower half of the bottle, functioning as a funnel. A roof was placed above these traps to protect them from rain entering the traps. Traps were emptied fortnightly over a period of six months (April until September 2002). The species needed for further genetic research were frozen in separate tubes and stored at -80°C.

Because of its more stringent habitat conditions, *C. problematicus* was absent from a large part of these forests (Table 1 and chapter 6). Plots wherein both species are examined are restricted to Buggenhout, Gasthuis, Meerdaal and two sampling plots in the Soignes forest that are separated 7.6 km from each other i.e. Soignes RK and Soignes P. Genetic data for C. problematicus in these forest plots are given in chapter 6. Time since isolation of the Kolenwoud remnants from the former Soignes forest are derived from literature and maps (Van der Ben 1992, Goemare 1998, Baeté et al. 2002). The degree of urbanization was estimated as described in chapter 2.

Fig. 1 – Geographic distribution of studied *Carabus violaceus* populations in the region of Brussels (Belgium) indicated in white and red. Red circles indicate where both *C. violaceus* and *C. problematicus* were genotyped. Plot numbers are given, full names are given in Table 1. Inset map shows the location of the sampling area in central Belgium, forests are presented in green.

Table 1 - Information on forest fragments and measures of genetic diversity at seven microsatellite loci in 19 *Carabus violaceus* populations.

[1] The percentage of urbanization was objectively calculated for each sample plot as described in chapter 2.

[2] Time since isolation is the period since the fragment was divided from the Soignes forest due to fragmentation processes.

[3] X indicates if the population is genotyped and compared to *C. violaceus*.

[4] The presence of the species in the forest plot is indicated with number 1, while 0 indicates the absence of the species in the forest plot.

Plot number	Forest plot	Area (ha)	% Urbanization[1]	Part of Kolenwoud	Time since isolation[2]	Presence C. problematicus[3,4]	Presence C. violaceus[4]	N	A	H_{exp}	H_{obs}	AR
1	**Soignes A**	4383.3	20.79	Yes	0	1	1	40	7.429	0.507	0.432	6.034
2	**Soignes F**	4383.3	0	Yes	0	1	1	40	6.714	0.498	0.414	5.488
3	**Soignes KL**	4383.3	4.554	Yes	0	1	1	40	6.429	0.477	0.464	5.646
4	**Soignes NV**	4383.3	0	Yes	0	1	1	40	7.143	0.541	0.482	6.010
5	**Soignes P**	4383.3	3.7	Yes	0	1 X	1	40	7.857	0.583	0.471	6.324
6	**Soignes RK**	4383.3	4.08	Yes	0	1 X	1	40	7.286	0.529	0.454	5.857
7	**Soignes rural**	4383.3	13.92	Yes	0	1	1	40	6.571	0.481	0.45	5.407
8	**Soignes**	4383.3	28.24	Yes	0	1	1	39	6.714	0.611	0.516	5.936
9	**Soignes Cam**	88.2	33.81	Yes	40	0	1	40	6.286	0.518	0.461	5.273
10	**Soignes Cam**	88.2	56.72	Yes	40	0	1	40	6.429	0.491	0.436	5.131
11	**Soignes Cam**	88.2	53.92	Yes	40	0	1	40	5.714	0.501	0.471	5.042
12	**Verrewinkel**	13.9	43.87	Yes	110	0	1	40	5.429	0.592	0.539	4.899
13	**Haller forest**	647.1	0.91	Yes	160	1	1	40	5.714	0.542	0.35	4.700
14	**Gasthuis forest**	38.9	22.35	Yes	510	1 X	1	40	6.143	0.528	0.454	5.240
15	**Kleet forest**	43.1	42.1	Yes	510	0	1	40	5.714	0.565	0.554	5.217
16	**Ronde forest**	5.4	56.08	Yes	510	0	1	40	4.714	0.396	0.332	3.810
17	**Meerdaal forest**	1575	0.78	Yes	1160	1 X	1	40	6.143	0.539	0.354	5.209
18	**Brugmanpark**	5.3	90.33	Yes		0	0					
19	**Dudenpark**	21.3	75.72	Yes		0	0					
20	**Buggenhout**	175.7	15.55	No	/	1 X	1	40	3.857	0.519	0.507	3.340
21	**Laarbeek forest**	37.9	15.54	No	/	0	1	40	4.143	0.463	0.425	3.785
22	**Dielgem forest**	15.2	74.48	No	/	0	0					
23	**Poel forest**	7.5	53.18	No	/	0	0					

Microsatellite typing

A total of seven microsatellite markers was used to genotype each individual. DNA was isolated from tissue samples (front legs) using Dneasy blood and tissue kit (Qiagen Inc.). The loci CV105136CMPG, CV109194CMPG, CV110036CMPG, CV109106CMPG and CV108106CMPG (Keller & Largiadèr, 2002) and the loci CV105216CMPG, CV108083CMPG, CV104481CMPG and CV108039CMPG (I. Keller, unpublished data) were isolated from *C. violaceus*. The PCR reactions were carried out as described in (Keller & Largiadèr, 2002). The PCR products were resolved on an ABI3130 automatic sequencer and alleles were sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and the Genemapper software of Applied Biosystems.

Statistical analyses

Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were first tested using a Markov chain method implemented in GENEPOP on the Web 4.0 (Raymond & Rousset, 1995, Rousset 2008) for each locus and for each population (n=19). Deviations from Hardy-Weinberg proportions were quantified by the unbiased estimator of Wright's inbreeding coefficient F_{IS} according to Weir & Cockerham (1984). Significance levels were adjusted for multiple testing using sequential Bonferroni corrections (Rice, 1989). To infer overall levels of population genetic differentiation, we computed the Weir & Cockerham's (1984) F_{ST} in GENETIX.

Average number of alleles per locus (A), expected mean heterozygosities (H_e) and observed mean heterozygosities (H_o) were calculated over all loci at each sampling location using the program POP100GENE 1.1.02 and allelic richness (AR) as a sample size independent measure of genetic diversity in FSTAT ver 2.9.3 (Goudet 2001) based on a minimum scored sample size of 28 individuals.

Diversity measures were subsequently correlated (Spearman rank order correlation) to forest area and time since isolation. To test if there is a significant difference in diversity measures between the populations of the fragments and the populations within the large Soignes forest, we used the two-tailed, non-parametric test implemented in FSTAT ver 2.9.3 (Goudet 2001) with 10000 permutations. To identify recent reduction in effective population size, we performed a heterozygosity excess test implemented in BOTTLENECK 1.2.02 (Piry et al. 1999) for each population under the three models SMM (stepwise mutation model), TPM (two phase model) and IAM (infinite allele model).

We used the population assignment test implemented in the program STRUCTURE to assign beetles to a cluster or population based on their genotypes without regard to where the specimens were collected (Pritchard et al., 2000). Allele frequencies were assumed correlated and Markov chains were run for 1000000 iterations, with a burn-in of 250000 generations. We used the admixture model, appropriate for populations that are expected to experience gene flow. Analyses were conducted with the number of clusters (K) set from 1 to 14, according to the number of fragments, to determine the likely number of clusters representative of the data (Pritchard et al. 2000).

An isolation-by-distance model (IBD) (Slatkin, 1993) was used to assess the correlation between genetic and geographic distances for all populations as well as for both the 8 plots originating from the different fragments as the 11 plots within the Soignes forest. Significance was evaluated in GENEPOP with a Mantel test (Mantel, 1976) with 10000 permutations (Raymond & Rousset, 1995; Rousset, 2008) using (F_{ST}/1-F_{ST}) against the natural logarithm of geographic distance.

To assess the relative importance of forest area, time since isolation and geographical isolation on the genetic structure, we further used a hierarchical Bayesian linear model implemented in GESTE v.2.0 (Foll & Gaggiotti, 2006) as described in chapter 6. The most appropriate model was selected by means of Bayes Factors. Analyses were run for 300.000 generations, of which the first 50.000 generations were treated as burn-in period and its values discarded for parameter estimation.

In order to compare the levels of differentiation between *C. violaceus* and *C. problematicus* into detail, we used the program GENETIX (Belkhir et al. 2004) to calculate the overall as well as pairwise levels of differentiation among the five fragments wherein both species are present. Also the overall differentiation and the pairwise population Dest values (Jost 2008) were calculated in SMOGD (Crawford 2009). This latter diversity measure also accounts for differences in allelic diversity compared to more traditional differentiation measures.

7.4 Results

In total, genetic data could be obtained from a total of 759 *C. violaceus* specimens that originated from the 19 different sampling plots

Intrapopulation genetic diversity of C. violaceus

The null hypothesis of Hardy-Weinberg equilibrium was not rejected in any population and none of the loci were in linkage disequilibrium in any of the populations. Therefore, further analyses were performed applying standard methods of population genetics.

We observed a total of 80 alleles at 7 microsatellite loci ranging from 3 to 17 alleles per locus (Appendix 6). The mean numbers of alleles (A), expected average heterozygosity (H_e), observed average heterozygosity (H_o) and allelic richness based on 28 individuals (AR) averaged to 6.128, 0.520, 0.451 and 5.176 respectively (Table 1). Two-tailed permutation tests revealed that allelic richness (P=0.0002) is higher in the populations from the continuous Soignes forest as compared to the fragments. BOTTLENECK analyses revealed no excess of heterozygosity, after Bonferonni correction, in any of the populations. None of the correlation tests to forest area and time since isolation turned out to be significant.

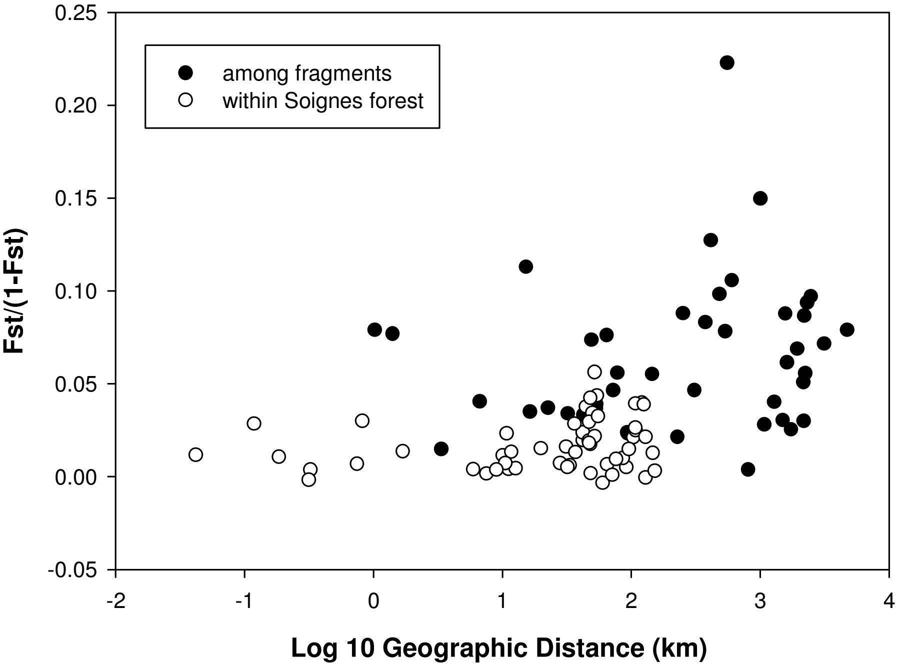

Fig. 2 - Relationship between geographic (log km) and genetic (F_{ST} /(1- F_{ST})) distance for all populations of *Carabus violaceus*. Open circles represent comparisons of populations from the large contiguous Soignes forest and filled circles represent population comparisons from the different forest fragments.

Table 2 - Pairwise F_{ST} (Weir & Cockerham) based on 7 microsatellite loci

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*																		
2	0.056	*																	
3	0.072	0.033	*																
4	0.051	0.015	0.047	*															
5	0.127	0.078	0.150	0.098	*														
6	0.079	0.025	0.030	0.030	0.087	*													
7	0.094	0.077	0.074	0.079	0.223	0.088	*												
8	0.069	0.037	0.055	0.041	0.083	0.040	0.113	*											
9	0.047	0.003	0.016	0.017	0.092	0.014	0.058	0.029	*										
10	0.039	0.028	0.041	0.023	0.108	0.041	0.035	0.054	0.014	*									
11	0.062	0.024	0.047	0.039	0.088	0.028	0.056	0.035	0.007	0.029	*								
12	0.085	0.024	0.013	0.038	0.088	0.009	0.084	0.025	0.006	0.038	0.020	*							
13	0.097	0.023	0.021	0.037	0.106	0.004	0.076	0.034	0.007	0.034	0.024	-0.003	*						
14	0.088	0.010	0.046	0.030	0.056	0.007	0.105	0.040	0.016	0.044	0.030	0.015	0.011	*					
15	0.054	0.003	0.030	0.010	0.059	0.007	0.093	0.023	0.005	0.033	0.020	0.007	0.012	0.004	*				
16	0.059	0.012	0.025	0.024	0.067	0.014	0.073	0.016	0.001	0.021	0.005	-0.001	0.002	0.013	0.004	*			
17	0.101	0.022	0.040	0.044	0.118	0.022	0.110	0.053	0.029	0.056	0.042	0.030	0.018	0.013	0.022	0.025	*		
18	0.091	0.012	0.029	0.034	0.061	0.011	0.099	0.039	0.010	0.040	0.026	0.003	0.005	0.002	0.007	0.004	0.021	*	

Genetic structure of C. violaceus

Overall degree of differentiation among 19 populations was moderately low (F_{ST} = 0.038, P=0.0189) and STRUCTURE assigned all of the individuals to a single population, with ln Pr(data|K) reaching its maximum for K=1. Pairwise F_{ST} estimates are represented in table 2. A Mantel test between geographic and genetic distance over all populations, revealed a significant IBD pattern (*N* = 19; R= 0.434, P = 0.02). When analyzing the fragments and the contiguous forest separately, Mantel tests showed a nearly significant correlation between genetic distances and geographic distances for the 11 plots within the Soignes forest (*N* = 11, R= 0.230, P=0.050), but turned out not to be significant in an analyses with only the forest fragments (*N* = 8; R=0.242, P=0.199) (Fig 2).

The linear model approach in GESTE (Foll & Gaggiotti, 2006) did not support a model that included geographic positioning of the fragments, time since isolation or forest area. Hence, the model with the highest probability only included the intercept, indicating that there is no clear relation between population specific F_{ST} values and any of the environmental factors we used in the analyses.

Genetic differentiation of C. violaceus compared to C. problematicus

We calculated the level of overall differentiation for *C. violaceus* and *C. problematicus* with the traditional differentiation measures and observed a total overall F_{ST} of respectively 0.040 and 0.120. Estimated measures of Dest gave a slightly higher measure of respectively 0.055 and 0.159. This shows that the level of differentiation appears to be a threefold lower in *C. violaceus* as compared to *C. problematicus* for populations living in the same fragments. Pairwise estimates of F_{ST} ranged from 0.012 to 0.101 for *C. violaceus* and from 0.014 to 0.152 for *C. problematicus.* Pairwise Dest estimates ranged from 0.015 to 0.101 for *C. violaceus* and from 0.034 to 0.303 for *C. problematicus* (Table 3)

Table 3 - Pairwise F_{ST} (Weir & Cockerham) and pairwise Dest (Jost) for *C. violaceus* and *C. problematicus* for forest fragments wherein both species occur.

Population	Population	F_{ST} *C. prob*	F_{ST} *C. viol*	Dest *C. prob*	Dest *C. viol*
1	2	0.152	0.056	0.302954	0.085644
	6	0.065	0.079	0.133831	0.100538
	16	0.077	0.059	0.153093	0.083069
	17	0.083	0.101	0.177405	0.127644
2	6	0.072	0.025	0.227262	0.035238
	16	0.068	0.012	0.211564	0.01491
	17	0.06	0.022	0.208509	0.022852
6	16	0.014	0.014	0.034035	0.015281
	17	0.015	0.022	0.048471	0.032737
16	17	0.007	0.025	0.048471	0.026152

7.5 Discussion

We used 7 microsatellite markers to investigate the genetic diversity, population genetic structure and gene flow among populations of the forest generalist ground beetle *C. violaceus* in forests in the region of a highly human populated region Brussels. We sampled in 8 fragments from different size as well as 11 plots within one contiguous large forest. In general, a lower allelic richness and a higher degree of differentiation was observed in the smaller remnants as compared to the plots in the contiguous forest. Furthermore, we compared part of these results to a former study on the forest specialist ground beetle *C. problematicus* (chapter 6) for plots where both species are present.

Forest area, history and degree of isolation

The different separated fragments became isolated fragments 100 to 1000 years ago accompanied by forest loss. Considering the low dispersal abilities of *C. violaceus*, it is likely that among these populations, gene flow became restricted as the number of neighboring populations declined dramatically. This is reflected in the lower values of allelic richness in the fragments as compared to the plots from the large contiguous Soignes forest. Keller et al. (2003) also demonstrated that the allelic richness in the smallest fragment, isolated by a highway, was lower than the rest of the study area. According to Lowe et al. (2003) allelic richness is the most valuable indicator of the genetic condition of the population, as it reflects population ability to adapt to ecological perturbations. Nevertheless, STRUCTURE analyses indicated that all populations constitute a single panmictic population wherein populations are still exchanging genes. One possible explanation for this can be found in a threshold F_{ST} value of STRUCTURE to detect differentiation between clusters (Latch et al. 2006). Indeed, the overall differentiation observed for *C. violaceus* approaches this value (F_{ST} =0.038). Second, if the decrease in genetic diversity is mainly due to the loss of rare alleles, while the overall allele frequencies are not changed substantially by genetic drift, it is unlikely that STRUCTURE will identify different clusters.

Pairwise F_{ST} among plots within the Soignes forest is lower compared to pairwise F_{ST} among populations originating from different fragments. This indicates that contemporary gene flow is more restricted among populations from different fragments. However, one needs to be cautious to interpret this result as evidence for restricted dispersal among populations from different fragments as the average geographic distance among these populations is evidently higher compared to populations from the same fragment. Indeed, our IBD analysis indicated that even within a single continuous fragment IBD patterns can emerge and suggests an equilibrium between drift and gene flow in the contiguous Soignes forest as a consequence of a continuous distribution, but restricted dispersal (Wright 1943, Slatkin 1993). However, given geographic distance, differentiation among populations from different fragments appeared to be slightly higher, indicating that populations from separate fragments are more strongly differentiated. Hence, these populations encounter other barriers to gene flow, than distance alone, indicated by their higher differentiation degree and lower diversity indices.

Other studies also demonstrated a reduced gene flow and reduced genetic diversity as a consequence of isolation (Knutsen et al. 2000, Segelbacher et al. 2003, Williams et al. 2003, Sumner et al. 2004, Keyghobadi et al. 2005, Noël et al. 2007, Biedrzycka & Konopinski 2008) or a low matrix quality between woodland patches (Lange et al. 2012) which can form barriers to dispersal.

Unfortunately, the linear model approach in GESTE did not support a model including effects of forest area or time since isolation. Keller et al. (2003) demonstrated that a habitat has to be quite small, i.e. a few hectares, for genetic factors to become important in the long-term survival of *C. violaceus*, but random demographic processes, common in insects (Thomas 1990) can cause local extinctions as well. Despite the low overall differentiation degree as compared to other microsatellite studies on ground beetles (Brouat et al. 2003, Keller et al. 2003, Dhuyvetter et al. 2005, Drees et al. 2008, Lagisz et al. 2010, chapter 6) and the assignment of all populations to one panmictic population, indicating enough gene flow, our results indicate that this forest generalist species is slightly affected by anthropogenic habitat deterioration and fragmentation. This may have significant consequences for surviving populations and raises concern for specialist species that are even more threatened.

Comparison of population differentiation between C. violaceus and C. problematicus

The lower estimated differentiation level (F_{ST} and Dest) for *C. violaceus* compared to *C. problematicus* seems to reflect a weaker genetic differentiation between populations of this forest generalist species as compared to forest specialists. This implicates lower gene flow between populations of the specialist species as compared to the generalist species and is in accordance to the study of Brouat et al. (2003). Ground beetles can be significantly structured, even on a very small geographic scale (Brouat et al. 2003, Keller & Largiadèr 2003, Garnier et al. 2004). Intrinsic dispersal capabilities of both species however, are very comparable (Thiele 1977), thus movement of this species is probably hampered by another factor. Since

C. problematicus has very specific habitat requirements and is dependent on ancient (Desender et al. 1999) relatively large forest (i.e. not smaller than ~30 ha, chapter 3 and 6), it is probably not able to cross the intervening matrix between two fragments and suffers therefore more from isolated forest patches, as compared to *C. violaceus*. A low differentiation level as found for *C. violaceus*, even in a fragmented landscape, can point towards a population that is well adapted to their actual distribution pattern and is not necessarily prone to extinction (Habel el at 2009).

Our results are consistent with the studies where increased levels of gene flow are associated with improved dispersal ability (Peterson & Denno 1998, Bohonak 1999). Even at a small spatial scale, genetic differentiation patterns of both species seem to reflect effects of forest fragmentation.

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7.8 Appendix

Appendix 5

Presence-absence surveys

Presence of *C. violaceus* was checked in 23 forest plots within 13 different forest fragments. In part of these plots we surveyed over a total period from 1 March to 19 November 2002 as described in Gaublomme et al. (2008). For the forests Meerdaal, Haller, Buggenhout and 7 plots in the Soignes forest we already disposed over a dataset previously collected by Desender.

C. violaceus was present in 19 of the 23 suited sampled forest plots (Table 1). Eleven of these plots were within the one large contiguous Soignes forest, from which three were located in the northern part of the Soignes forest that is cut off from the rest of the forest by two major roads and located in a highly built-up area. *C. violaceus* was absent in a total of four studied plots i.e. three located in a highly urbanized environment (>70% urbanized) and in the Poelbos fragment (around 50% urbanized). *C. problematicus* was only present in 6 of the 23 sampled forest plots (Table 1).

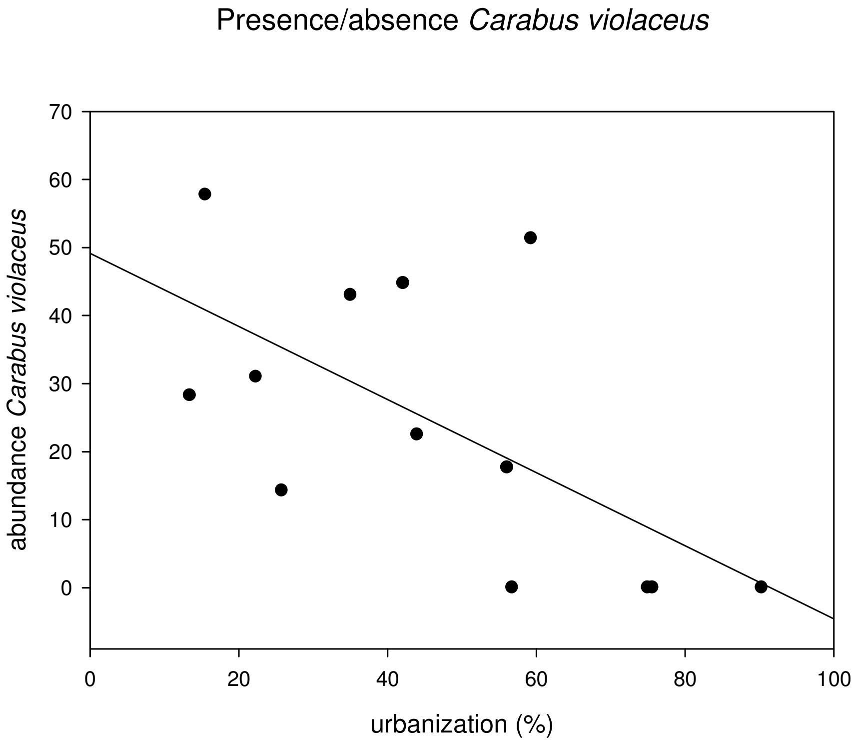


Fig. 1 – Relationship between urbanization (%) and abundance of *Carabus violaceus*.

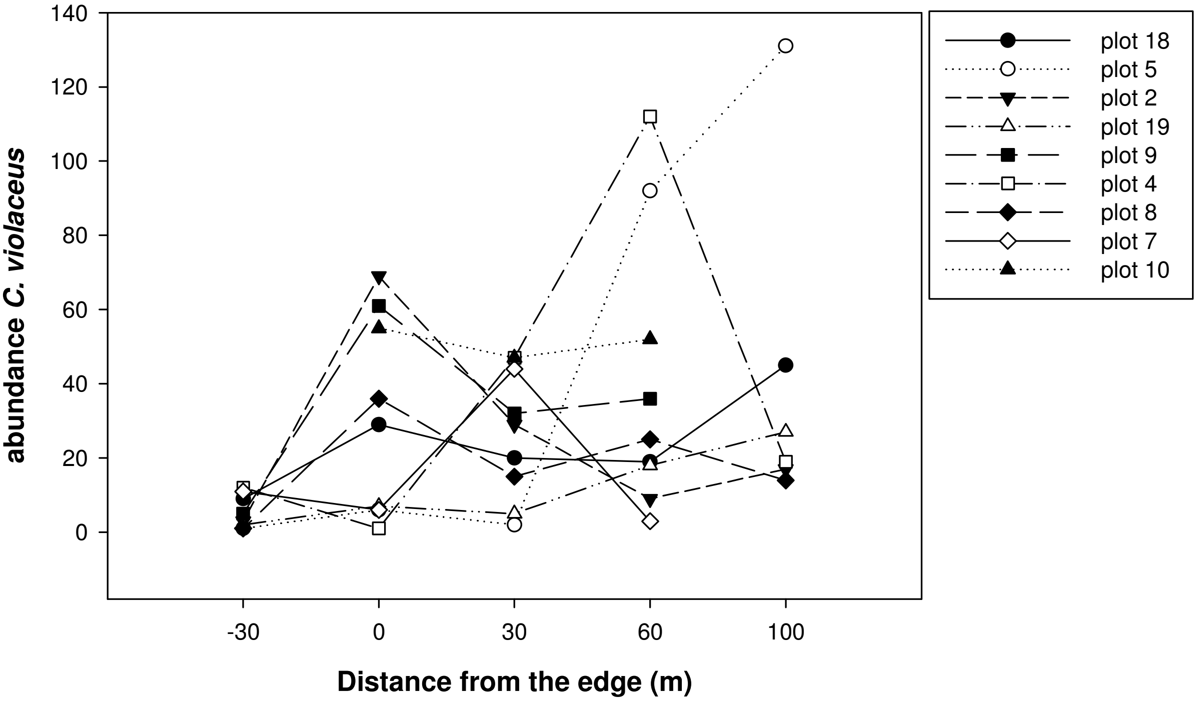


Fig. 2 – Abundance of *Carabus violaceus* along the transect from outside the forest to the forest interior (m) in nine different plots

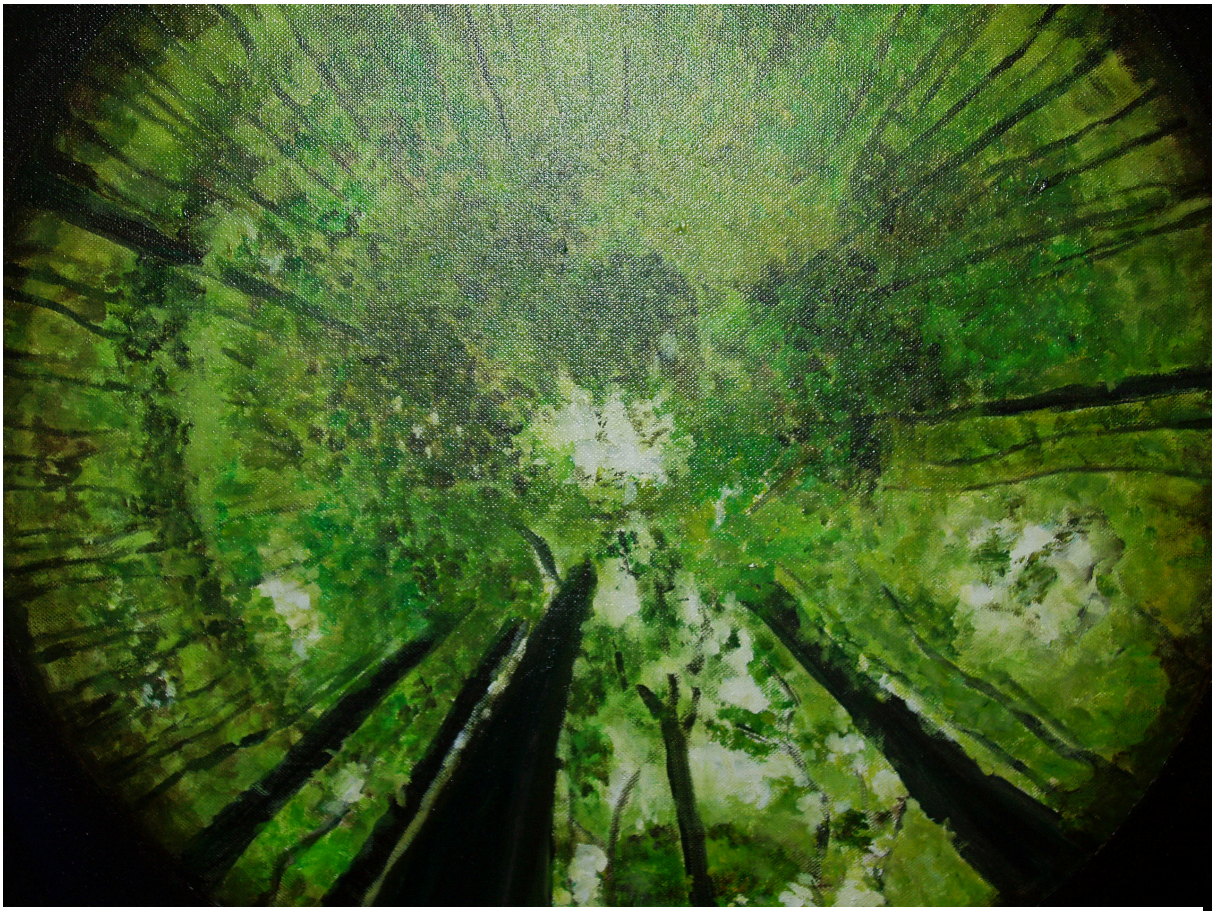
Appendix 6 - Allele frequencies from seven microsatellite loci in 19 populations.

Locus	Allele	Buggenhout	Gasthuis forest	Haller forest	Kleet forest	Laarbeek forest	Meerdaal forest	Ronde forest	Verrewinkel	Soignes Cam X	Soignes Cam U	Soignes Cam Cru	Soignes A	Soignes F	Soignes KL	Soignes NV	Soignes P	Soignes RK	Soignes rural	Soignes suburban
105216																				
Gene Number		80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	78
Allele Number		5	7	6	6	3	5	5	6	6	8	7	7	7	6	8	8	7	8	7
	0	0.08	0.03	0.25	0.00	0.13	0.15	0.00	0.00	0.00	0.03	0.00	0.00	0.05	0.03	0.08	0.08	0.08	0.03	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.01	0.00	0.04	0.00	0.00	0.01	0.00	0.03	0.03	0.04	0.03	0.01	0.01	0.03	0.03	0.03	0.08	0.03
	3	0.08	0.20	0.04	0.19	0.56	0.20	0.06	0.31	0.24	0.18	0.30	0.30	0.26	0.30	0.28	0.29	0.06	0.28	0.35
	4	0.00	0.06	0.00	0.00	0.00	0.00	0.14	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
	5	0.19	0.09	0.23	0.10	0.00	0.01	0.09	0.06	0.10	0.10	0.04	0.11	0.03	0.01	0.06	0.05	0.03	0.06	0.09
	6	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.01	0.01	0.03	0.05	0.06	0.01	0.01	0.11	0.01	0.01
	7	0.15	0.04	0.04	0.01	0.00	0.00	0.00	0.13	0.08	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.04
	8	0.51	0.58	0.43	0.60	0.31	0.55	0.70	0.46	0.55	0.59	0.59	0.50	0.59	0.59	0.51	0.50	0.69	0.51	0.45
	9	0.00	0.00	0.03	0.06	0.00	0.00	0.00	0.03	0.01	0.06	0.01	0.01	0.01	0.00	0.03	0.04	0.01	0.03	0.04
Heterozygote Proportion		0.45	0.23	0.45	0.58	0.28	0.23	0.40	0.55	0.50	0.43	0.60	0.68	0.55	0.40	0.60	0.60	0.40	0.68	0.62
Gene Diversity		0.68	0.62	0.71	0.60	0.58	0.64	0.49	0.68	0.63	0.62	0.57	0.65	0.59	0.57	0.66	0.67	0.51	0.66	0.68
109106																				
Gene Number		80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	78
Allele Number		2	3	5	4	2	3	4	3	6	7	4	6	6	4	6	6	6	5	4
	0	0.00	0.00	0.03	0.00	0.00	0.03	0.08	0.00	0.03	0.03	0.00	0.05	0.10	0.00	0.00	0.05	0.03	0.03	0.03
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	2	0.59	0.36	0.34	0.53	0.28	0.34	0.58	0.31	0.41	0.54	0.41	0.26	0.26	0.25	0.34	0.34	0.16	0.25	0.31
	3	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.03	0.05	0.01	0.01	0.04	0.01	0.04	0.05
	4	0.00	0.04	0.00	0.00	0.00	0.00	0.09	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.11	0.00	0.00
	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.03	0.04	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
	7	0.41	0.60	0.58	0.43	0.73	0.64	0.26	0.60	0.51	0.31	0.54	0.59	0.55	0.71	0.61	0.55	0.68	0.66	0.62
	8	0.00	0.00	0.05	0.03	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.06	0.03	0.03	0.01	0.01	0.01	0.03	0.00
Heterozygote Proportion		0.68	0.60	0.63	0.55	0.50	0.38	0.38	0.53	0.58	0.70	0.48	0.53	0.43	0.48	0.50	0.48	0.53	0.43	0.54
Gene Diversity		0.49	0.51	0.56	0.55	0.40	0.49	0.60	0.54	0.57	0.62	0.55	0.59	0.62	0.43	0.52	0.59	0.51	0.50	0.53

105136																				
Gene Number		80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	78
Allele Number		2	2	4	2	2	3	2	2	2	2	2	3	3	2	2	3	3	2	2
	0	0.00	0.00	0.13	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.03	0.05	0.00	0.00	0.08	0.03	0.00	0.00
	1	0.78	0.74	0.76	0.76	0.71	0.89	0.91	0.78	0.84	0.85	0.81	0.85	0.86	0.85	0.81	0.73	0.81	0.84	0.68
	2	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.23	0.26	0.09	0.24	0.29	0.06	0.09	0.23	0.16	0.15	0.19	0.13	0.09	0.15	0.19	0.20	0.16	0.16	0.32
Heterozygote Proportion		0.40	0.38	0.23	0.43	0.33	0.13	0.18	0.35	0.28	0.25	0.33	0.20	0.18	0.30	0.28	0.30	0.33	0.33	0.44
Gene Diversity		0.35	0.39	0.40	0.37	0.42	0.21	0.16	0.35	0.28	0.26	0.31	0.27	0.25	0.26	0.31	0.43	0.32	0.28	0.44
108083																				
Gene Number		80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	78
Allele Number		3	3	3	4	4	4	2	5	5	4	7	7	5	6	3	9	8	4	7
	0	0.13	0.00	0.00	0.08	0.00	0.00	0.03	0.00	0.00	0.03	0.03	0.03	0.00	0.00	0.00	0.08	0.03	0.03	0.21
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.01	0.00	0.01	0.00	0.00	0.01	0.04	0.00	0.06	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.00
	4	0.86	0.98	0.95	0.81	0.95	0.88	0.98	0.66	0.94	0.93	0.88	0.89	0.86	0.91	0.95	0.76	0.84	0.90	0.69
	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.01	0.00	0.00
	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.01	0.03	0.01	0.00	0.01
	8	0.01	0.01	0.04	0.06	0.01	0.00	0.00	0.09	0.01	0.00	0.00	0.03	0.04	0.01	0.00	0.05	0.03	0.06	0.04
	9	0.00	0.00	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
	10	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.04	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	11	0.00	0.01	0.01	0.00	0.00	0.05	0.00	0.00	0.03	0.00	0.01	0.01	0.04	0.00	0.00	0.01	0.01	0.00	0.01
	12	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.01	0.04	0.01	0.03	0.04	0.00	0.03	0.01	0.00	0.00
	13	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00
Heterozygote Proportion		0.03	0.05	0.05	0.23	0.10	0.20	0.00	0.60	0.13	0.10	0.20	0.18	0.23	0.15	0.10	0.30	0.23	0.13	0.21
Gene Diversity		0.24	0.05	0.10	0.33	0.10	0.23	0.05	0.53	0.12	0.14	0.23	0.21	0.25	0.17	0.10	0.41	0.30	0.19	0.48
109194																				
Gene Number		80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	78
Allele Number		8	13	9	11	9	12	9	9	10	11	11	11	12	13	13	14	12	12	12
	0	0.05	0.03	0.13	0.00	0.00	0.13	0.03	0.00	0.08	0.05	0.03	0.05	0.03	0.00	0.05	0.00	0.03	0.03	0.00
	1	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	2	0.01	0.04	0.13	0.00	0.00	0.00	0.00	0.06	0.05	0.01	0.04	0.09	0.01	0.03	0.04	0.03	0.00	0.06	0.05
	3	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00
	4	0.30	0.04	0.04	0.00	0.05	0.04	0.01	0.00	0.10	0.05	0.14	0.00	0.00	0.04	0.01	0.04	0.03	0.01	0.08
	5	0.23	0.31	0.39	0.29	0.15	0.29	0.19	0.31	0.34	0.30	0.20	0.39	0.44	0.24	0.35	0.38	0.35	0.33	0.35
	6	0.19	0.06	0.01	0.16	0.23	0.09	0.00	0.09	0.06	0.01	0.06	0.06	0.06	0.06	0.14	0.09	0.03	0.14	0.08
	7	0.00	0.09	0.00	0.01	0.01	0.04	0.11	0.00	0.04	0.00	0.04	0.05	0.06	0.13	0.06	0.05	0.04	0.09	0.10
	8	0.00	0.06	0.03	0.09	0.00	0.00	0.10	0.01	0.00	0.04	0.09	0.04	0.05	0.09	0.09	0.08	0.11	0.05	0.03

	9	0.18	0.25	0.16	0.16	0.11	0.08	0.36	0.23	0.21	0.25	0.21	0.16	0.23	0.23	0.13	0.16	0.21	0.14	0.14
	10	0.03	0.04	0.01	0.04	0.05	0.05	0.00	0.04	0.06	0.13	0.08	0.05	0.03	0.05	0.03	0.09	0.06	0.06	0.06
	11	0.00	0.04	0.11	0.04	0.04	0.04	0.11	0.00	0.05	0.01	0.00	0.00	0.01	0.00	0.00	0.03	0.09	0.03	0.01
	12	0.00	0.01	0.00	0.09	0.00	0.00	0.00	0.23	0.01	0.05	0.01	0.04	0.04	0.03	0.03	0.01	0.01	0.00	0.03
	13	0.00	0.03	0.00	0.04	0.30	0.06	0.08	0.01	0.00	0.10	0.11	0.01	0.01	0.05	0.05	0.03	0.03	0.06	0.06
	14	0.00	0.00	0.00	0.04	0.06	0.08	0.00	0.03	0.00	0.00	0.00	0.06	0.00	0.03	0.03	0.00	0.00	0.01	0.01
	15	0.00	0.00	0.00	0.05	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.01	0.01	0.00	0.00	0.00
	16	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.01	0.00	0.00	0.00
Heterozygote Proportion		0.85	0.75	0.60	0.88	0.78	0.70	0.75	0.83	0.75	0.80	0.83	0.65	0.75	0.88	0.78	0.83	0.73	0.83	0.90
Gene Diversity		0.80	0.83	0.79	0.85	0.82	0.88	0.80	0.80	0.82	0.82	0.87	0.81	0.75	0.87	0.83	0.82	0.81	0.84	0.84
104481																				
Gene Number		80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	78
Allele Number		3	8	7	7	4	8	6	5	8	8	4	7	6	6	7	6	7	8	7
	0	0.00	0.05	0.30	0.08	0.00	0.28	0.03	0.03	0.10	0.03	0.00	0.05	0.03	0.00	0.08	0.20	0.23	0.05	0.03
	1	0.06	0.05	0.11	0.25	0.00	0.13	0.03	0.10	0.09	0.03	0.05	0.14	0.11	0.16	0.18	0.05	0.08	0.05	0.04
	2	0.35	0.08	0.03	0.06	0.36	0.06	0.06	0.15	0.04	0.18	0.01	0.00	0.00	0.13	0.13	0.06	0.06	0.03	0.13
	3	0.00	0.10	0.01	0.13	0.14	0.03	0.00	0.00	0.13	0.14	0.16	0.08	0.13	0.15	0.08	0.10	0.19	0.16	0.09
	4	0.59	0.44	0.50	0.36	0.48	0.43	0.83	0.65	0.56	0.59	0.78	0.69	0.66	0.48	0.46	0.58	0.40	0.56	0.60
	5	0.00	0.09	0.03	0.01	0.00	0.03	0.03	0.00	0.01	0.01	0.00	0.03	0.05	0.01	0.01	0.01	0.01	0.03	0.06
	6	0.00	0.18	0.00	0.11	0.00	0.05	0.04	0.08	0.06	0.03	0.00	0.01	0.03	0.08	0.08	0.00	0.04	0.11	0.05
	7	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.00	0.03	0.03	0.00	0.03	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Heterozygote Proportion		0.58	0.65	0.25	0.68	0.68	0.53	0.25	0.43	0.55	0.53	0.30	0.48	0.48	0.70	0.53	0.38	0.48	0.50	0.54
Gene Diversity		0.54	0.76	0.65	0.78	0.63	0.73	0.32	0.55	0.65	0.61	0.38	0.51	0.54	0.71	0.73	0.62	0.75	0.65	0.61
108039																				
Gene Number		80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	78
Allele Number		4	7	6	6	5	8	5	8	7	5	5	11	8	8	11	9	8	7	8
	0	0.00	0.05	0.30	0.00	0.00	0.25	0.03	0.18	0.08	0.05	0.00	0.08	0.10	0.00	0.08	0.10	0.03	0.00	0.28
	1	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
	2	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.05	0.00	0.00
	4	0.25	0.08	0.04	0.08	0.06	0.00	0.00	0.13	0.01	0.10	0.06	0.04	0.01	0.03	0.08	0.09	0.05	0.01	0.06
	5	0.64	0.66	0.58	0.71	0.84	0.58	0.79	0.50	0.65	0.79	0.60	0.69	0.71	0.81	0.59	0.66	0.70	0.86	0.46
	6	0.00	0.16	0.04	0.08	0.01	0.03	0.01	0.01	0.16	0.05	0.14	0.03	0.04	0.09	0.08	0.09	0.06	0.05	0.09
	7	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.04	0.03	0.01	0.00	0.06	0.05	0.03	0.04	0.00	0.06	0.03	0.00
	8	0.00	0.03	0.00	0.01	0.00	0.01	0.13	0.00	0.00	0.00	0.15	0.00	0.00	0.01	0.04	0.01	0.00	0.03	0.03
	9	0.00	0.01	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.03	0.01	0.03	0.01	0.04
	10	0.00	0.00	0.01	0.04	0.00	0.05	0.00	0.09	0.05	0.00	0.05	0.03	0.03	0.01	0.01	0.01	0.00	0.01	0.01
	11	0.00	0.00	0.04	0.09	0.00	0.01	0.05	0.05	0.00	0.00	0.00	0.03	0.05	0.00	0.05	0.01	0.03	0.00	0.03
	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	13	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00

	14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	15	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heterozygote Proportion		0.58	0.53	0.25	0.55	0.33	0.33	0.38	0.50	0.45	0.25	0.58	0.33	0.30	0.35	0.60	0.43	0.50	0.28	0.39
Gene Diversity		0.53	0.53	0.58	0.48	0.29	0.61	0.37	0.70	0.55	0.37	0.60	0.52	0.48	0.33	0.64	0.54	0.50	0.26	0.70



Painting of fish eye photo for light measurements in the forest by Luc Gaublomme © Eva Gaublomme

Chapter 8

GENERAL DISCUSSION

Chapter 8

General discussion

8.1 Overview of the main results

In a first phase, we investigated the effects of forest patch size, distance to the forest edge and degree of urbanization on the distribution of carabid beetles in the highly fragmented old beech forests situated in the densely built-up area of Brussels in 2002. To achieve this, we sampled 13 forest plots in 10 different forest fragments, ranging in size from 5.27 to 4383 ha. The beetles were captured using transects from 30 m outside the forest (=matrix) to 100 m inside the forest. Effects of urbanization, forest area and forest edge were evaluated on total abundance and species richness, abundance of habitat affinity groups and individual abundance of ten species with different habitat requirements. The influence of the matrix type, measured as the degree of urbanization (%), had an important negative effect on all ground beetles irrespective whether the response was measured as total abundance, total species richness, abundance of individuals assigned to different habitat preference group or at species level. The only exception to this pattern is the open landscape species *Bembidion properans*, which was favored by the effects of urbanization in the surrounding matrix. Disentangling the effects of forest area and edge effects was less straightforward since interactions with urbanization played a significant role. Moreover the magnitude and direction of the effect of the different factors differed when analyzed on total abundance level, total species richness level, abundance of the habitat affinity group level and single species level. Species richness and abundance proved not good measures to investigate the effects of fragmentation. Disturbances may favor widespread and abundant generalist species, leading to an increase in richness (Niemelä 2001). Total abundance is strongly driven by abundance of the most common species, such as *Nebria brevicollis*, and therefore conceals the effects on more vulnerable species. From a conservation perspective, however, strongest interest lies in the abundance of specialized forest species. This also stresses the importance of analyzing at the species level instead of generalizing beyond species level in order to really understand the different effects of fragmentation (chapter 2).

Clearer patterns were observed when effects of fragmentation and forest edge distance are investigated at the level of species composition and species turnover. Although it is generally assumed that edge effects and forest area have significant interacting effects on populations in remaining habitat fragments (Ewers et al. 2007, Didham et al. 1998b, Barbosa & Marquet 2002), we found that the strength of edge effects was independent of increasing habitat area. Fragment area seemed far more important and small fragments only contained an impoverished forest beetle community rather than species invading from the surrounding area. Even for small fragments, edge effects apparently did not penetrate to the center of the forest but remained restricted to the border of the forest. The most clear consequence of a decreasing forest patch size, was that forest specialist species disappeared from smaller fragments, suggesting that demographic and environmental stochasticity are the most important drivers of species composition in ancient forest fragments (chapter 3).

In a next series of studies, we attempted to assess the population genetic consequences on two ground beetle species that are particularly bound to well-developed forest habitat. We used microsatellites that have the advantage of being locus specific, co-dominant and highly polymorphic. They are of particular interest to ecologists because they are one of the few molecular markers that allow researchers to gain insight into fine-scale ecological questions (Selkoe & Toonen 2006). Unfortunately, a given pair of microsatellite primers rarely works across broad taxonomic groups especially in arthropods, and so primers are usually developed anew for each species (Glenn & Schable 2005). Therefore we developed microsatellite primers for the forest specialist ground beetle *Carabus problematicus* (chapter 4) .

Since this specialist ground beetle might be at risk in small populations, we tested the effect of taking a tissue sample (a leg of the second pair of legs) and a haemolymph sample for genetic studies on the mortality rate of this species and another comparable large specialist species. Mortality rate did increase after haemolymph sampling but not after tissue sampling. Both sampling techniques could be used for allozyme and microsatellite genotyping (chapter 5). One disadvantage of the sampling technique for allozyme genotyping is the need for immediate storage in liquid nitrogen when sampling directly in the field.

Many ground beetles preferring forest as habitat are characterized by a low dispersal ability and, hence, are more susceptible to the consequences of habitat fragmentation i.e. decreasing forest patch size and isolation, compared to species living in more dynamic habitat types. We investigated the effect of patch area and isolation by genotyping 29 populations of the forest specialist *Carabus problematicus* in 21 different forests scattered throughout Belgium. Reduction of forest area led to a high genetic differentiation and a low genetic diversity, demonstrating that genetic drift was more pronounced in smaller populations of this forest specialist species. High differentiation levels among different forest fragments as compared to those situated within one large forest demonstrated a strong decrease in gene flow when corrected for geographic distance. This indicated that the lack of long distance dispersal combined with the stringent habitat requirements of the specialist species hampered gene flow and as such threatens the survival of this species in the long term (chapter 6).

The species *C. violaceus*, although also clearly bound to forest habitat and not capable of long distance dispersal by flight, is less stringent in its habitat requirements and indeed also found in the intervening matrix. We therefore suspected it to be less sensitive to the effects of fragmentation compared to *C. problematicus*. We sampled 19 locations within the region of Brussels, 11 populations within the contiguous Soignes forest and 8 populations in smaller forest fragments that were once connected to the Soignes forest and thus shared a common history. We found a lower allelic richness and higher differentiation level among the fragments as compared to the different populations within the large Soignes forest. In line with our expectations, genetic differentiation was a threefold higher in *C. problematicus* compared to *C. violaceus*, indicating a more restricted gene flow in the former. It appears that the forest generalist species is able to cross the matrix insofar this matrix is not too hostile (i.e. >70% of urbanization, chapter 7 appendix Fig 1).

8.2 Effects of forest fragmentation on ground beetles in an urban context

Based on the theory of island biogeography (MacArthur & Wilson 1967), it can be expected that fragment area and isolation are one of the most important factors determining the consequences of habitat fragmentation on biodiversity. However, more recently, additional effects of fragmentation are considered i.e. (i) deteriorating within-patch quality due to the creation of forest edge at the cost of forest interior and (ii) the effect of matrix habitat (Gascon & Lovejoy 1998).

In our study on forest fragmentation in an urban context, we attempted to focus on these four factors simultaneously.

Fragment area

A reduction of habitat area as a consequence of fragmentation leads to concomitant changes in the size of local populations (MacArthur & Wilson 1967) and is called the species-area relationship as described by Pimm et al. (1995). In our study, decreasing forest patch area had a strong influence on ground beetle communities (chapter 3), primarily by its negative effect on the preservation of forest specialist species (chapter 3 and 6). Small fragments contained ground beetle communities that lack highly specialized forest species and were favored by generalist species (chapter 2). Since forest area per se was the most important variable explaining differences in species composition among all plots located within the forest fragments (chapter 3), extinction of forest specialist species was most likely caused by demographic and environmental stochastic effects. Specialist butterflies were also more affected by habitat area reduction than generalist species (Krauss et al. 2003) and the most specialist forest ground beetle species occurred only in continuous forest (Halme & Niëmela 1993). Small populations restricted to a small forest patch are more likely to go extinct than large populations (Hanski 2002).

In the smaller fragments we observed a lower allelic richness as compared to the populations in the large contiguous Soignes forest for the forest generalist species *C. violaceus* (chapter 7). This erosion of genetic diversity as a consequence of drift was also reflected in the positive relationship between fragment area and all measures of genetic diversity in populations of *C. problematicus* (chapter 6). A reduced population size enhances the risk of diversity loss due to drift processes (Taylor 2003, Hedrick 2005) and generates populations that are genetically more distinct from each other, thus with a higher differentiation rate (Williams et al. 2003, Sumner et al. 2004, Noël et al. 2007, Biedrzycka & Konopinski 2008). This was clearly shown in the populations of the forest specialist species, *C. problematicus* where smaller forests exhibited a considerably higher genetic differentiation compared to large forests (chapter 6).

Isolation

Besides a reduced genetic variability as a consequence of drift in smaller populations (Saunders et al. 1991, Frankham 1995, Westemeier et al. 1998), fragmentation also leads to restricted gene flow among populations, resulting in higher levels of genetic differentiation among populations (Hutchinson & Templeton 1999, Frankham et al. 2002, Segelbacher et al. 2003, Delaney et al. 2010). Fragments become isolated because of fragmentation and decreased rates of movement among the remaining fragments results in a reduced gene flow

(Knutsen et al. 2000). An isolation by distance (IBD) analysis for *C. problematicus* showed virtually no differentiation among populations within the fragments, but revealed strong genetic differentiation among populations of different fragments when correcting for geographic distance (chapter 6). For populations of the forest generalist species we found IBD within the large contiguous Soignes forest indicating an equilibrium between genetic drift and gene flow, but not between the different fragments (chapter 7). However, when comparing genetic differentiation between the forest generalist and the forest specialist species, differentiation was a threefold higher in the forest specialist species, implicating lower levels of gene flow between the populations of the forest specialist species (chapter 7). These higher levels of differentiation were in accordance to the species specific habitat requirements of the forest specialist species, which makes it impossible to recolonize a fragment once it is extirpated. To the contrary, a forest generalist species is more likely to traverse the surrounding matrix to colonize other fragments and this was reflected in a much lower genetic differentiation between the populations (chapter 7). In accordance with other studies, these results show that responses to habitat fragmentation are indeed species specific and depend on the degree of specialization (Brouat et al. 2003), individual habitat requirements and dispersal abilities as well as stochastic events (Wiegand et al. 2005). Therefore we need to be careful with conclusions derived from the study of one specialist and one generalist only.

Edge effects

In a fragmented landscape, there is an abrupt contrast between the communities residing within a habitat patch and those found in the surrounding matrix (Heliolä et al. 2001, Martin & Major 2001, Basset et al. 2008). Not surprisingly, this was observed in our study where community analyses revealed a highly distinct community inside and outside the forest fragment (chapter 3). Along the edge to interior gradient, an increasing number of specialist species and a decreasing number of open landscape species was found towards the interior of the forest (chapter 2). More surprisingly however, was the limited effect of edges within the forest, as shown by a very restricted scale on which these effects responded, i.e. only the forest border. Hence, species composition of the forest interior plots was only marginally different from those found in plots at the edges of the forest patches. An obscuring factor in our dataset could be that 100 m into the forest is not far enough to reach the interior, but when looking at Fig 4 in chapter 3 we clearly observe that an asymptote is reached at 60 m from the forest border. This suggests that edge effects on ground beetles could be less pronounced than generally expected (Ewers et al. 2007, Fletcher et al. 2007, Banks-Leite et al. 2010). Even in small fragments where it is suspected that edge effects are stronger because of the higher edge to area ratio (Murcia 1995, Didham et al. 1998 a, b, Ries & Sisk 2004, Ewers & Didham 2006, Ewers et al. 2007), we demonstrated that the strength of edge effects appeared independent of decreasing area (chapter 3).

This result is strikingly different from recent studies i.e. Ewers et al. 2007 where edge effects drive the observed variation in community structure that was only superficially linked to habitat area. Small fragments showed little or no edge effect because of the absence of forest-like conditions and as such they were dominated by a matrix-dwelling fauna that could penetrate the entire area of the fragment. Our CA analyses however revealed that the community found in small fragments was very different from the communities found in the large Soignes fragment and that this was primarily due to the absence of typical forest specialists rather than due to an invasion of matrix-dwelling species. The communities found in the forest plots at the border of the fragment were moreover much more similar to those found in the forest interior than those found in the adjacent matrix. However, we need to stress that one should be cautious in generalizing these results as only one single large fragment was included. The only large forest included in our set-up is Soignes forest where four replicates were taken and can therefore be interpreted as pseudo-replicates. Nevertheless it needs to be stressed that forest plots (i.e. Soignes extra and Soignes urban), only separated from the large Soignes forest by two major roads, already resulted in the absence of forest specialist species. Moreover, small fragments only contain forest generalist species and not species invading from the surrounding matrix. Hence, these small fragments still retain some value for conservation and should be protected. Future studies however, could circumvent this possible bias by also including other larger forests that were part of the historic Kolenwoud, such as Haller forest or Meerdaal forest.

Matrix effects

In recent fragmentation studies more emphasis has been put on the influence of the landscape matrix on individuals, populations and communities (Prevedello & Vieira 2010), especially in human-dominated landscapes (Laurance 2008, Delaney et al. 2010). In this context, the Globenet project was set up in 2000 to assess and compare landscape changes related to human activities on a global scale. Carabid beetles in forests were sampled by pitfall trapping across urban-rural gradients to examine the impact of urbanization on the responses of carabid beetles in different cities around the world (Niemelä et al. 2000). To date, results were published from nine locations in various forest ecosystems in Europe, Canada and Japan and a general conclusion was that urbanization favors open landscape species while forest species declined in a more urban area (Niemelä & Kotze 2009, Magura et al. 2010, Tothmeresz et al. 2011). In chapter 2 we also demonstrated the degree of specialization to have an important effect on the susceptibility of the species towards urbanization, because specialist species show little flexibility to cope with a changing environment. In our study, however, we also included the effects of distance to the forest edge and forest area and calculated the interaction between urbanization and these two factors. We found an interacting effect of forest area and matrix type, measured as the degree of urbanization. If the matrix was highly urbanized, total species richness appeared higher in small fragments compared to large fragments. The most likely explanation is a more heterogeneous matrix and therefore more diverse ground beetle fauna in an urban area as compared to a more rural surrounding area. Unfortunately, invading species from this urban matrix were generalist species, able to survive in many habitats and are not of primary concern for the conservation of a forest patch in which mainly forest species are the focus for preservation.

As previously mentioned, we also found the matrix to have an effect on the dispersal of the forest specialist species *C. problematicus* as this matrix formed a barrier to dispersal, causing higher differentiation levels as a lack of gene flow.

8.3 Use of ground beetles as indicators for fragmentation effects

The results from this thesis support the notion that ground beetles are good indicators for forest fragmentation in an urban environment as they clearly show distinct responses to various effects of forest fragmentation. Moreover, sampling beetles from various habitats is far less demanding compared to vertebrate sampling and extensive invertebrate datasets can easily be obtained. Yet, we already demonstrated that care should be taken at which level the effects are assessed, and prior knowledge of the habitat affinities of the species is a stringent requirement to accurately address the effect of presumed deteriorating human factors. Combining individual species responses with community analyses provided the most reliable results. Using this approach, we were able to demonstrate that forest generalist and forest specialist species showed a different response to the effects of habitat fragmentation. Analyzing upon abundance of the habitat affinity group also generates valuable information, since most species belonging to the same group react in a common way on the different effects of habitat fragmentation.

8.4 Conservation implications

In addition of being indicators of fragmentation, ground beetles themselves can be the target for conservation, because some of the typical forest species are severely threatened in Flanders (Desender et al. 2008). Problems resulting from habitat fragmentation are the problems encountered by a modified environment of reduced area, increased isolation and new ecological boundaries (Ewers & Didham 2006). In an urban environment, it is almost impossible to enlarge existing patches. Fragments, large enough and still containing very vulnerable forest specialist species (i.e. *Carabus problematicus* was never recorded in forests smaller than 30 ha, chapter 2) are therefore extremely important to be protected from further negative human interference. If forests are large enough, i.e. Soignes forest, to maintain the existing populations, the surrounding matrix is of minor importance. Small fragments though, should be surrounded by a high quality matrix to at least enable species lacking the most stringent habitat requirements to disperse (Fahrig 2001, Öckinger & Smith 2008, Desender et al. 2010, Prevedello & Vieira 2010). *C. violaceus*, a species with no very stringent habitat requirements was able to move through the matrix if this matrix is not too hostile. When the urbanization degree was larger than 70% this species disappeared from the forest patch (chapter 2, chapter 7 appendix). Furthermore, the effects of edge were only weak which makes small patches also valuable for protection. Within the context of the SLOSS (several large or single small) debate in conservation (Zimmerman & Bierregaard 1986), data from our study are quite straightforward in particular with respect to forest specialists and are in accordance with a previous study on ground beetles (Lövei et al. 2006). For the conservation of forest specialist species, it is crucial to have a large fragment (chapter 3 and 6)
whereas for the conservation of forest generalist species, several small patches are sufficient if there is a possibility to use the matrix to reach these different fragments (chapter 3 and 7). This implies that the quality of the matrix is sufficiently good which facilitates dispersal among fragmented patches and thus further supports the importance of one large as the best option. In this way conservation of forest specialist species and forest generalist species is assured, so increasing matrix quality (Prevedello & Vieira 2010) can be complementary to habitat restoration and re-creation in fragmented landscapes.

One of the most popular approaches for maintaining populations and conserving biodiversity in fragmented landscapes is to retain or create corridors that connect otherwise isolated habitat patches. Movements of different taxa with broadly different life histories and functional roles are directed by corridors (Haddad et al. 2003). In our study, we demonstrated that edge effects are only moderate and restricted to the border of the forest. This has direct consequences for the usefulness of corridors for ground beetles in improving the connectivity between different forest fragments. Since the impact of edge effects appeared of relatively minor magnitude on the community assembly at 30m into the forest, corridors have not to be wider than around 50 meters to be functional for forest species. The quality of the corridor is however important in order to be useful for forest specialist species. However, the extent to which corridors increase connectivity for an organism may depend not only on the corridor itself, but also on the composition of the surrounding matrix (Baum et al. 2004). Therefore, it is recommended to create a low-resistance matrix which increases the number of forest species that can use the corridor to move from one fragment to another. Nevertheless, corridor effectiveness is species-specific (Beier & Noss 1998) and might not be sufficient for all forest specialist species. However, Eggers et al. 2010 demonstrated that the forest specialist *Carabus auronitens* used the semi-open corridors (a mixture of heath land and woodland vegetation) to reach forest patches. The advantage here is that the corridors themselves do not break up another habitat, here heath land, whereby the corridor itself would also create a barrier to the typical heath land fauna, if it would consist of woodland only. Drees et al. (2011) and Matern et al. (2011) showed the effectiveness of increased connectivity by means of hedges and afforestation for a forest specialist and forest generalist ground beetle respectively.

Translocation of extirpated species as a conservation tool can be used, but unfortunately reintroductions are sometimes performed by volunteers without prior adequate research (i.e. the beaver *Castor fiber* and the spider *Eresus sandaliatus* in Belgium). Before introduction, habitat requirements and availability for the focal species have to be examined (Maunder 1992) and conservation measurements should be taken to improve the habitat according to the species' requirements. The ecological factors that contributed to the extirpation of the species have to be considered and these factors have to be eliminated to reach a possible successful outcome. Moreover, the impacts of reintroduction are particularly hard to predict and can be very harmful to other species, so care should be taken and a proper legislation is recommended.

Practical guidelines for conservation

- If possible enlarge existing patches
- Give priority to patches still containing specialist species
- If possible improve the quality of the surrounding matrix, especially in environments containing several smaller fragments
- Smaller patches are also worth protecting, since edge effects are only weak
- Create good quality corridors
- Translocation of species is only useful after a thorough study of the habitat and the focal species in particular

8.5 Future directions

Landscape genetics and dispersal

From the results obtained in this study, we concluded that matrix type is important to consider in order to ensure movement of ground beetles between the different patches and, hence, in advancing gene flow. Decreased interpatch dispersal due to barriers in the landscape are however not easy to detect, but will eventually also lead to spatially structured populations. Knowledge on how landscape structure affects dispersal, genetic variability and hence population persistence is very scarce (Diekötter et al. 2007, Matern et al. 2008). In the growing field of landscape genetics, population genetic data are combined with landscape data to understand how the landscape elements impact the genetic structure of populations (Manel et al. 2003). The ability of a species to move across the landscape is critical for maintaining populations (Fahrig 2003). The species investigated in detail in this study are not able to disperse by flight and indeed showed a high tendency to depend on matrix type in order to disperse. However, little is known on how ground beetles effectively use the landscape. So far, most studies on insect dispersal were based on mark-recapture techniques, but movement models also have been developed for different ground beetles (Jopp & Reuter 2005). Therefore landscape genetics combined with movement modelling seem very promising in helping to understand the fate of these vulnerable populations (Storfer et al. 2007, Diekötter et al. 2010).

Separating contemporary and historical influences on genetic structure

Estimating the effects of anthropogenic fragmentation on population genetic structure is difficult, since genetic structure is also subjected to historic complex geological and climatic changes. The effects of these historical events could overwhelm the population genetic changes due to recent anthropogenic fragmentation, since they all contributed to current patterns of genetic diversity. To separate the effects of historical and contemporary processes on population genetic structure, we can use genealogically informative markers such as DNA sequences (Templeton 1998). In this way it is not necessary to investigate a small geographic area to be sure of a mutual history, but it remains possible to enlarge the study area and still be able to distinguish between historical processes and more recent events.

In a recent study on rattlesnakes (Chiucchi & Gibbs, 2010) contemporary versus historical gene flow was compared by using assignment tests versus coalescent techniques to estimate migration rates over few versus many generations (Beerli & Felsenstein 2001, Wilson & Rannala 2003) and additionally measures of genetic variation were used to detect changes in population size over different timescales (Cornuet & Luikart 1996).

These two different methods seem very promising in disentangling historical from contemporary gene flow.

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Summary

The process of habitat fragmentation leads to a reduction in the total amount of natural habitat, resulting in highly heterogeneous landscapes composed of isolated fragments of suitable habitat that are embedded in a matrix of often unsuitable habitat. Consequences of fragmentation include creation of habitat patches of varying size and degree of isolation. The theory of island biogeography suggested that species richness is a function of island size and degree of isolation and metapopulations dynamics theory suggests that populations remaining in remnant patches depend on immigration for their long-term persistence. Reduction in remnant patch size following fragmentation can result in fragments of a size that are insufficient to sustain viable populations and increased isolation of patches can pose serious risks to overall long-term populations stability. More recently however, the focus has been moved to other additional factors of habitat fragmentation. Deteriorating fragment quality due to the creation of forest edge at he cost of forest interior and the effect of matrix habitat. In this thesis we attempt to increase our knowledge on these four effects of fragmentation on ground beetles in beech forest in an urban context with special focus on the highly urbanized Brussels region. In a first phase we investigated the effects of forest patch area, distance to the forest edge and degree of urbanization on community and species level. In a second part we revealed the population genetic consequences of forest patch area and matrix effects on two wingless ground beetle species, that are particularly bound to forest habitat, but in a different degree.

In chapter 2 we compared ground beetles from a range of different forest fragments along an urbanization gradient in Brussels' forests. We investigated if the degree of urbanization affected ground beetles and if there was an interacting effect with forest patch area and distance to the forest edge. These effects were studied on three different levels: total community, habitat affinity group and individual species. The effects of urbanization, forest area and edge effects slightly influence total species richness and abundance but appeared to have a major effect on ground beetle assemblages through species specific responses. Our results show the importance of differentiating between habitat affinity, especially habitat generalists versus specialists and merely the quantity of species represented in human-dominated areas.

In chapter 3 we attempt to disentangle the effect of smaller population size as reflected by smaller forest patches and increasing edge effects of small and fragmented patches. Although species assemblages within the forest were highly distinct compared to those sampled outside the forest for all fragments, species turnover was less pronounced within forest fragments indicating only weak edge effects. The magnitude of edge effects was also not significantly differing between large and small fragments. However, larger differences in species composition were observed with respect to fragment size, wherein highly specialized species persisted only in large fragments. Local extinction processes, rather than edge effects, are by far more important to explain the absence of specialized forest species in this fragmented woodland complex.

Chapter 4 is a methodological chapter where we described the development of species specific microsatellite markers for the forest species *Carabus problematicus*.

In chapter 5 we tested the influence of two non-destructive sampling techniques on the survival of two ground beetles and checked the usefulness of the obtained samples in genotyping based on enzyme electrophoresis and microsatellites.

In chapter 6 we investigated the effects of decreasing patch size and increasing isolation on a typical forest species with low dispersal ability. We genotyped individuals from 29 sampling locations throughout Belgium of the species *C. problematicus* from 21 different forest fragments. A high degree of genetic differentiation among fragments was observed, with populations from smaller forests being considerably more differentiated and characterized by a lower genetic diversity. We also observed a higher genetic differentiation among fragments as within fragments irrespective of geographic distance. This indicated that patch fragmentation rather than geographic distance is the ultimate factor hampering gene flow. The inability of this species to cross the landscape matrix is probably reducing gene flow and may therefore pose a serious threat for the persistence in fragmented habitat patches.

In chapter 7 we investigated 19 different populations of a forest generalist ground beetle, situated in the region of Brussels of which 8 populations were found in smaller fragments and 11 populations were caught within the large contiguous Soignes forest. For these *C. violaceus* populations we observed a lower genetic diversity and a higher differentiation level in the fragments as compared to the plots in the contiguous forest, indicating reduced mobility and gene flow. We also compared the consequences of habitat fragmentation on the forest specialist species *C. problematicus* as described in chapter 6 to the more generalist forest species *C. violaceus*, with less stringent habitat requirements, but disposing over equal dispersal capacities. Differentiation was significantly higher in *C. problematicus* and indicated, together with the presence of this species in the matrix habitat, an elevated level of gene flow for the forest generalist due to less stringent habitat requirements.

Based on the study of these ecological and genetic effects of urbanization and fragmentation on ground beetles in Belgian beech forests, we can conclude that forest fragment size and the possibility of the species to function as a metapopulation are still the main drivers of fragmentation effects. This latter is indeed strongly depending on the quality of the patch and the ability of the species to cross the intervening matrix between the different forest patches. Edge effects, on the other hand seem less important, than described in recent studies. Besides general effects of fragmentation much is depending on the ability of the individual species to cope with these landscape changes. Generally forest specialist species will suffer more from the detrimental effects, since there habitat requirements are more stringent and the ability to use the intervening matrix is very low, which makes colonization extremely difficult. To maintain the existing populations in our Belgian forests it is important to keep the fragments as large as possible.

Samenvatting

Het proces van habitat fragmentatie resulteert in een afname van het natuurlijk habitat, wat leidt tot een verhoogde heterogeniteit van het landschap. Dit landschap is samengesteld uit geïsoleerde fragmenten omgeven door een matrix van vaak ongeschikt habitat. De gevolgen van fragmentatie zijn dus het ontstaan van habitat stukken van verschillende oppervlaktes en een verschillende graad van isolatie. De eiland biogeografie theorie zegt dat soortenrijkdom afhankelijk is van de grootte van het fragment en de mate van isolatie en de metapopulatie dynamica theorie suggereert dat populaties in overgebleven fragmenten afhankelijk zijn van immigratie voor hun voortbestaan op lange termijn. Een afname in oppervlakte als gevolg van fragmentatie kan leiden tot te kleine fragmenten waarin een populatie niet meer leefbaar is en dit samen met een verhoogde mate van isolatie kan de overleving van populaties op lange termijn enorm op de proef stellen. Meer recent is de focus bij onderzoek naar habitat fragmentatie nu ook verschoven naar andere bijkomende factoren. Een afname in de kwaliteit van het habitat door het creëren van bosranden ten koste van het binnenste van het bos en de effecten van omliggend habitat. In dit doctoraatsonderzoek willen we deze vier factoren van fragmentatie onder de loep nemen met betrekking tot de effecten op loopkevers in beukenbossen en dit in een urbane omgeving met speciale aandacht voor de sterk verstedelijkte regio rond Brussel. In een eerste luik onderzochten we de effecten van bosoppervlakte, afstand tot de bosrand en de mate van verstedelijking op gemeenschapsniveau alsook op individueel niveau. In een tweede luik onderzochten we de populatiegenetische gevolgen van oppervlakte en matrix effecten op twee vleugelloze loopkevers, die beide afhankelijk zijn van boshabitat.

In hoofdstuk 2 vergeleken we loopkevers uit bosfragmenten van verschillende grootte langsheen een urbanisatie gradiënt in de Brusselse bossen. We bekeken of de mate van verstedelijking invloed had op de loopkevers en of er interactie was tussen bosoppervlakte en afstand tot de rand. Deze effecten werden bestudeerd op drie verschillende niveaus: gemeenschapsniveau, per groep gebonden aan een bepaald habitat, soortsniveau. De effecten van urbanisatie, bosoppervlakte en randeffecten beïnvloedden soortenrijkdom en –abundantie in beperkte mate, maar hadden een grote invloed op de totale gemeenschap ten gevolge van soortspecifieke reacties. Onze resultaten toonden duidelijk aan dat het belangrijk is om een onderscheid te maken tussen de verschillende groepen gebonden aan een bepaald habitat, voornamelijk het verschil tussen algemene bossoorten en gespecialiseerde bossoorten in een verstoorde omgeving.

In hoofdstuk 3 probeerden we de effecten van bosoppervlakte en randeffecten van elkaar te onderscheiden. Ondanks de grote verschillen tussen soortengemeenschappen binnen en buiten het bos, was er slechts een kleine verandering merkbaar binnen de gemeenschap als we kijken van de rand naar het centrum van het bos. Dit duidt op gering waarneembare randeffecten die ook niet groter werden naarmate het fragment kleiner was. Toch waren er zeer grote verschillen merkbaar tussen grote en kleine fragmenten, waarbij gespecialiseerde bossoorten enkel voorkwamen in de grote fragmenten. Plaatselijke uitstervingsprocessen, eerder dan randeffecten, blijken dan ook de oorzaak te zijn van het verdwijnen van die gespecialiseerde bossoorten in dit gefragmenteerd bossencomplex.

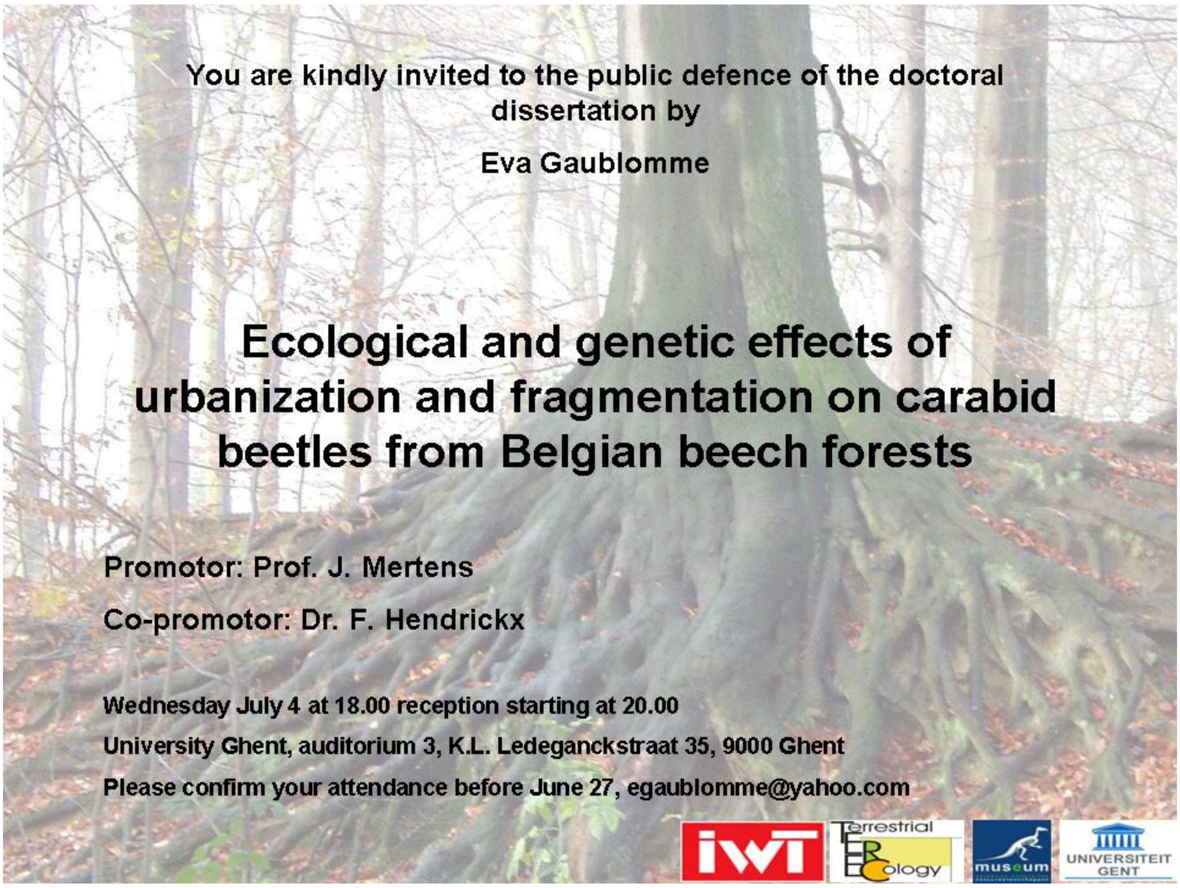
Hoofdstuk 4 is een methodologisch onderzoek waarin we soortspecifieke microsatelliet primers ontwikkelden voor de bossoort *Carabus problematicus*.

In hoofdstuk 5 onderzochten we twee verschillende staalname technieken van DNA met zo weinig mogelijk impact op het individu. We bekeken de overleving van twee soorten na staalname en checkten de bruikbaarheid van de stalen voor enzym elektroforesen en microsatelliet onderzoek.

In hoofdstuk 6 bekeken we de effecten van een verkleinde oppervlakte en een verhoogde mate van isolatie van het bosfragment op de bossoort *C. problematicus,* die een lage dispersie capaciteit heeft. We genotypeerden 29 staalnamepunten verspreid over geheel België afkomstig uit 21 verschillende fragmenten. We noteerden een hoge mate aan genetische differentiatie tussen de verschillende fragmenten, met populaties uit kleine fragmenten opvallend meer gedifferentieerd en gekenmerkt door een lagere genetische diversiteit. We zagen ook een hogere genetische differentiatie tussen fragmenten vergeleken met binnen fragmenten onafhankelijk van de geografische afstand. Dit wijst erop dat fragmentatie, eerder dan geografische afstand aan de oorsprong ligt van een verlaagde genetische uitwisseling. De moeilijkheid voor deze soort om de tussenliggende matrix tussen de bossen te overbruggen is waarschijnlijk de oorzaak van deze verlaagde genetische uitwisseling en kan daardoor een grote bedreiging vormen voor de overleving in een gefragmenteerd gebied.

In hoofdstuk 7 onderzochten we 19 populaties van een algemene bosloopkever in de omgeving van Brussel. We bekeken 11 populaties binnen eenzelfde aaneengesloten groot bos, het Zoniënwoud en 8 populaties afkomstig van verschillende kleinere bosfragmenten. Deze *C. violaceus* vertoonden een hogere differentiatie en een verlaagde genetische diversiteit in de fragmenten ten opzichte van het groot aaneengesloten bos, wat terug wijst op een verlaagde genetische uitwisseling ten gevolge van beperkingen in verspreiding van deze soort tussen de fragmenten. We vergeleken tevens ook de gevolgen van habitat fragmentatie tussen de twee verschillende loopkevers, *C. violaceus* en *C. problematicus* die verschillen in gebondenheid aan het habitat. De mate van differentiatie bleek veel hoger te liggen bij de bosspecialist en dit samen met een totale afwezigheid van deze soort buiten het bos wijst erop dat bosgeneralisten, die wel buiten het bos voorkomen, toch een verhoogde genetische uitwisseling hebben tussen fragmenten door hun minder strenge eisen ten opzichte van hun omgeving.

Uit deze studie op de ecologische en genetische effecten van urbanisatie en fragmentatie, kunnen we concluderen dat bosgrootte en de mogelijkheid van een soort om zich te hanteren als metapopulatie heel belangrijk blijven bij habitat fragmentatie. Dit laatste is echter sterk gerelateerd aan de kwaliteit van het fragment en de mogelijkheid van de soort om tussenliggend habitat tussen de fragmenten te overbruggen. Randeffecten, in onze studie, bleken minder belang te hebben. Behalve deze algemene effecten van habitat fragmentatie, hangt zeer veel af of de individuele soort kan omgaan met deze veranderingen in het landschap. Algemeen zullen gespecialiseerde bossoorten meer negatieve invloed ondervinden, omdat ze strenge eisen stellen aan hun omgeving en ze daardoor minder gebruik kunnen maken van het tussenliggend habitat, wat kolonisatie enorm bemoeilijkt. Om de overblijvende populaties in onze Belgische bossen te besschermen is het dus zeer belangrijk om de resterende fragmenten zo groot mogelijk te houden.



You are kindly invited to the public defence of the doctoral dissertation by

Eva Gaublomme

Ecological and genetic effects of urbanization and fragmentation on carabid beetles from Belgian beech forests

Promotor: Prof. J. Mertens

Co-promotor: Dr. F. Hendrickx

Wednesday July 4 at 18.00 reception starting at 20.00

University Ghent, auditorium 3, K.L. Ledeganckstraat 35, 9000 Ghent

Please confirm your attendance before June 27, egaublomme@yahoo.com

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U wordt vriendelijk uitgenodigd op de publieke verdediging van het doctoraal proefschrift door

Eva Gaublomme

Ecologische en genetische effecten van urbanisatie en fragmentatie op loopkevers in Belgische beukenbossen

Promotor: Prof. J. Mertens

Co-promotor: Dr. F. Hendrickx

Woensdag 4 juli om 18u met aansluitend receptie vanaf 20u

Universiteit Gent, auditorium 3, K.L. Ledeganckstraat 35, 9000 Gent

Graag aanwezigheid bevestigen voor 27 juni, egaublomme@yahoo.com