

Loss of genetic diversity and increased genetic structuring in response to forest area reduction in a ground dwelling insect: a case study of the flightless carabid beetle *Carabus problematicus* (Coleoptera, Carabidae)

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Abstract. 1. Old growth temperate broadleaved forests are characterised 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 a flightless and forest specialist beetle *Carabus problematicus* from 29 populations, sampled in 21 different forest fragments in Belgium, at eight microsatellite loci.

3. A high degree of genetic differentiation among fragments was observed, with populations from smaller forests being considerably more differentiated and characterised by a lower genetic diversity compared to those of larger forests.

4. A more detailed study on forest remnants of a former historic continuous woodland area revealed that population differentiation was high among, but not within remnants, irrespective of geographical distance. This suggests that patch fragmentation rather than geographical distance is the ultimate factor that hampers gene flow in this species.

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

Key words. Genetic differentiation, ground beetle, habitat fragmentation, isolation, microsatellites, population genetics.

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

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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 specialisation (Ewers & Didham, 2006) of the species under study. In particular, species with limited dispersal ability are expected to be the most vulnerable. 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, recolonisation 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 (Bloemers & Van Dorp, 1991; Verhulst, 1995; Honnay *et al.*, 2005). In Belgium, the transition of natural forests to agricultural land has 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; Desender *et al.*, 1999). Although habitat fragmentation is known to affect the long-term survival of many species, most studies in Belgium are conducted on plants (Bossuyt *et al.*, 1999; Hermy *et al.*, 1999; Honnay *et al.*, 1999). However, studies that investigated the population genetic effects of landscape fragmentation on animals have shown that landscape deterioration may strongly affect genetic structuring (Keyghobadi, 2007). In particular, species incapable of flight and specialised species that are unlikely to find sufficient suitable habitat patches for dispersal (Brouat *et al.*, 2004), 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, 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 urbanised areas, there is a strong need for such guidelines among policy makers, managers and conservation practitioners.

The forest 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. Because this ground beetle species is incapable of flight, long-distance dispersal 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 evaluate how this can be related to the factors forest area and geographical distance.

Materials and methods

Study species

Carabus problematicus Herbst, 1786 in our study region is a strictly nocturnal forest specialist beetle that is incapable of flight. Movement up to 70–75 m per night has been observed (Neumann, 1971; Rijnsdorp, 1980). Although it is capable of dispersal by walking, this species is not able to cross the forest surrounding matrix.

Study area and sampling

A total of 1221 beetles, distributed over 29 samples, were obtained 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 samples were taken (Table 1). About 40 beetles per sample 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 held depending on the difficulty to reach a sample of 40 individuals per sample.

Of particular interest was the Brussels Capital Region, with a range of four old growth forest fragments: Gasthuisbos, Rodebos, Meerdaalwoud and Soignes (Fig. 2). The historical ecology of these last forests is well documented (Van der Ben, 1992; Tack *et al.*, 1993; Verhulst, 1995). All fragments originated from the historic forest Kolenwoud (Table 1; Fig. 1), which was a mediaeval forest, covering the central part of Belgium with a total area of 12 000 ha. From the 12th century onwards, it was partly subdivided into smaller fragments and its total area reduced to 10 382 ha in the 16th century (Van de Velde, 1992). In the 18th century, the largest part, constituting

Table 1. Summary of the 29 *Carabus problematicus* samples, with forest fragment origin (forest fragment), size of the forest fragment in ha, sample size expressed as number of genotyped individuals (N) and measures of genetic diversity at seven microsatellite loci in 29 *C. problematicus* populations. Fragments indicated with (K) were part of the former Kolenwoud, measures of genetic diversity were mean number of alleles per locus (A), average observed heterozygosity (H_o), average expected heterozygosity (H_e) and estimated allelic richness summed over all loci for a subset of 26 individuals (AR).

Sample	Forest fragment	Fragment size (ha)	N	A	H_o	H_e	AR
Brak	Brakel	243	44	4.43	0.49	0.51	28.86
Brasin	Inslag	394	38	4.43	0.44	0.43	28.29
Bugg	Buggenhout	176	61	3.71	0.35	0.37	22.66
Buze	Buzenol	16 200	41	4.86	0.50	0.59	33.27
Chanly	Chanly	46 199	40	5.71	0.59	0.67	38.28
Fernelm	Fernelmont	168	29	3.29	0.44	0.50	22.92
Gasth	Gasthuisbos (K)	39	33	3.71	0.49	0.48	25.50
Har	Harentbeek	241	38	4.86	0.50	0.53	31.60
HVMuhl	Moelenbusch	25 000	39	5.14	0.53	0.54	32.93
HVRuhr	Ruhrbusch	15 310	39	5.00	0.53	0.57	33.09
Kalm	Withoefse Heide	70	38	4.14	0.43	0.45	26.51
Kluis	Kluisbos	274	39	4.29	0.40	0.47	28.46
Make	Bruinbos	82	54	4.14	0.46	0.51	25.60
Meerddr	Meerdaalwoud (K)	1575	36	5.71	0.57	0.58	37.58
MeerJ +60	Meerdaalwoud (K)	1575	40	6.29	0.54	0.56	39.24
MeerJ-40	Meerdaalwoud (K)	1575	40	5.71	0.57	0.58	36.49
MeerJ-60	Meerdaalwoud (K)	1575	40	6.14	0.55	0.57	39.39
Meerdkl	Meerdaalwoud (K)	1575	35	5.57	0.56	0.57	36.55
MeerO +60	Meerdaalwoud (K)	1575	41	5.43	0.50	0.56	35.34
MeerO-115	Meerdaalwoud (K)	1575	37	5.86	0.48	0.52	37.32
Ppame	Amerloo-Asbroek	152	39	4.43	0.37	0.47	29.29
Ppeer	Peerdsbos	280	31	4.00	0.42	0.44	27.22
Rode	Rodebos (K)	104	38	5.29	0.42	0.47	34.28
Roval	Rodebos (K)	104	36	5.29	0.45	0.47	34.99
Soree	Soree	30	48	4.57	0.49	0.52	29.41
Voer15	Veursbos	1031	37	4.14	0.47	0.47	28.00
Voer7	Alserbos	77	41	3.29	0.38	0.41	22.20
ZONP	Soignes (K)	5103	37	5.57	0.53	0.58	36.89
ZONRK	Soignes (K)	5103	36	5.14	0.57	0.58	34.28
Overall (\pm SE)				4.83 (\pm 0.17)	0.515 (\pm 0.01)	0.482 (\pm 0.01)	31.92 (\pm 5.44)

mostly of the current Soignes forest, had a total area of 10 800 ha (Lefèbvre, 1997). From 1843 until now the remaining Soignes forest retained its current area of 4383 ha and forms together with other remnants (Table 1) the last remains of the former Kolenwoud. A thorough sampling on a smaller scale was conducted here, so that several samples per fragment were taken: Soignes ($n = 2$), Gasthuisbos ($n = 1$), Meerdaal ($n = 7$) and Rodebos ($n = 2$; Table 1; Fig. 1). This detailed sampling in the Brussels Capital Region probably comprises most of the current Kolenwoud remnants in which the study species is still present (Gaublomme *et al.*, 2008).

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 of study species using the DNeasy blood and tissue kit (Qiagen Benelux, Venlo, the Netherlands). PCR reactions were carried out as described in Gaublomme *et al.* (2003) and Keller and Largiadèr (2002), and PCR products were resolved on an ABI3130 automated DNA sequencer (Applied Biosystems, Gent, Belgium) using an internal size standard (GS500Liz; Applied Biosystems). Microsatellite allele sizing and binning were conducted with GENEMAPPER v3.7 (Applied Biosystems).

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 sample ($n = 29$). Deviations from



Fig. 1. Geographical distribution of studied *Carabus problematicus* populations in Belgium. Forests are indicated in grey and sampling locations with triangles. The black border lines represent Belgian ecoregions. (a) Detail of the four Kolenwoud fragments with 12 sampled populations of *C. problematicus*. (b) For position and location see Fig. 3.

Hardy-Weinberg proportions were quantified by the unbiased estimator of Wright's inbreeding coefficient F_{IS} according to Weir and 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 sample ($n = 29$), genetic diversity was assessed as allelic richness (AR; El Mousadik & Petit, 1996) as a sample size independent measure of genetic diversity in FSTAT ver 2.9.3 (Goudet, 2001) and 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>). To test whether or not genetic diversity decreased with decreasing fragment size, these four diversity indices were correlated (Spearman rank order correlation coefficient) to log transformed forest area in STATISTICA 6.0. (StatSoft Inc., Hamburg, Germany, 1998).

To identify putative recent reductions in effective population size, we used the heterozygosity excess test implemented in BOTTLENECK 1.2.02 (Piry *et al.*, 1999) for

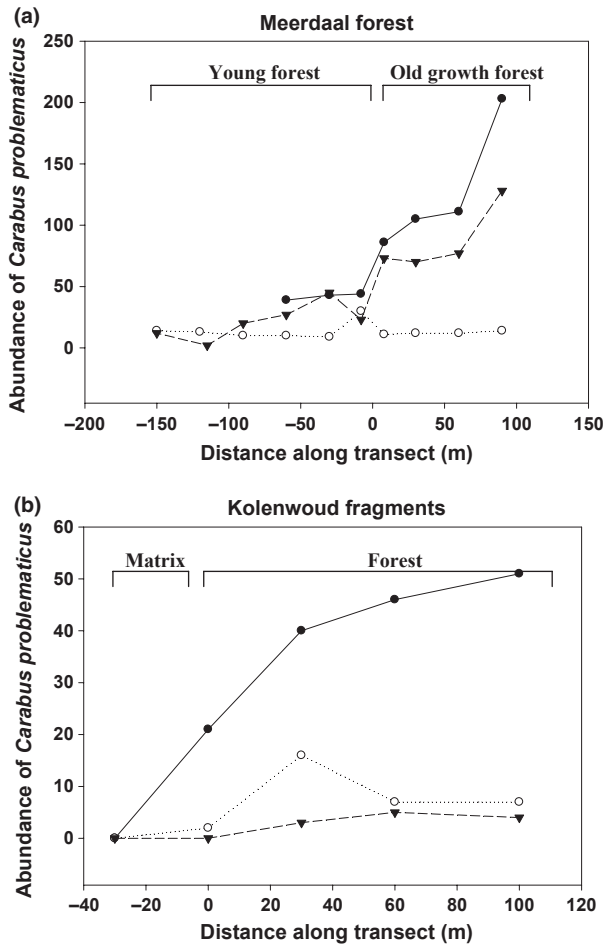


Fig. 2. Abundance of *Carabus problematicus* along transects (a) of young forest extending into old growth forest (b) from 30 m into the matrix to 100 m inside the forest fragment.

each sample ($n = 29$). This software simulates the coalescence process for loci at mutation-drift equilibrium, evolving under a two-phase model of mutation as recommended for microsatellites (Cornuet & Luikart, 1996) with 1000 iterations for significance testing using Wilcoxon signed rank tests.

Geographical distances between the sampling locations were obtained using ARCVIEW version 3.1 (ESRI, Redlands, CA, USA). 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 digitisation for the forest fragments Brugmanpark and Verrewinkelbos. An isolation-by-distance model (Slatkin, 1993) was used to assess the correlation between genetic and geographical distances among the fragments ($n = 21$). Significance was evaluated

in GENEPOP using a Mantel test (Mantel, 1976) with 10 000 permutations (Raymond & Rousset, 1995; Rousset, 2008) using $(F_{ST}/1-F_{ST})$ against the natural logarithm of geographical distance.

We subsequently focused on the spatially more restricted set of samples originating from the 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 among samples within fragments with the pattern observed among samples originating from different forest fragments. For this restricted set of samples, we also quantified the partitioning of genetic variation at different hierarchical levels that is among forests ($n = 4$); among samples 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, sample specific F_{ST} 's are estimated 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 a set of nested models to be entered that can then be compared based on their posterior model probabilities. By mean of this model, we tested if population specific F_{ST} 's are related to (i) the geographical location of the sampled fragments by means of the variables latitudinal and longitudinal distance (Lambert x-coordinate) (Foll & Gaggiotti, 2006) and (ii) area of forest fragment. We also included 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. To test if there is spatial autocorrelation in fragment size (i.e. if larger fragments tended to be spatially clustered), we quantified Moran's I and tested if it was significantly larger than zero.

Results

Carabus problematicus was never found outside the forest fragments along the sampled transects (Fig. 2a; Gaublonne *et al.*, 2008). Sampling a transect of old growth forest that persisted during the past 230 years (i.e. since the first systematic maps of de Ferraris appeared in 1770–1778), the abundance of *C. problematicus* declined drastically when sampling was extended into younger forest of 50–150 years old (Fig. 2b). This demonstrates that its specific habitat requirements, that is strictly bound to forest and preferably old growth forest, combined with its inability to disperse by flight, are the more determining factors that restrict dispersal among forest fragments.

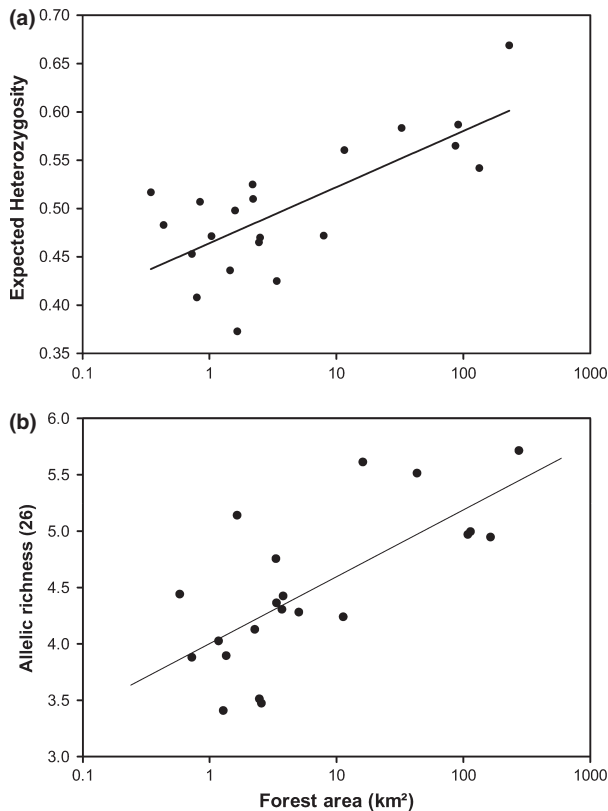


Fig. 3. Relationship between forest area and average expected heterozygosity (a) and allelic richness based on 26 individuals per population over all loci (b) in *Carabus problematicus*

Genetic diversity

The total number of alleles per locus ranged from 7 to 27. Eleven alleles (of a total of 89 alleles at seven loci) were present at all sampling sites, whereas 11 alleles were private and only found in 10 different samples, originating from seven different fragments that is Moelenbusch (one allele), Meerdaalwoud (four alleles), Soignes (one allele), Chanly (two alleles), Bruinbos (one allele), Withoefse heide (one allele) and Soree (one allele). The mean number of alleles (A), expected average heterozygosity (H_e), observed average heterozygosity (H_o) and AR based on 26 individuals (AR) across samples averaged to 4.83 (± 0.17 SE), 0.515 (± 0.01 SE), 0.482 (± 0.01 SE) and 31.92 (± 5.44 SE) respectively (Table 1). Locus P99 showed deviations from HWE and was withdrawn from further analyses. None of the loci were in linkage disequilibrium for any of the samples.

BOTTLENECK analysis revealed no excess of heterozygosity in any of the samples, thus, there were no traces of recent genetic bottlenecks. Inbreeding coefficients were not significantly different from zero in all samples and for all loci.

Forest fragment 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 AR ($n = 21$, $r = 0.704$; $P < 0.001$), indicating that samples from larger forest fragments were genetically more diverse.

Genetic differentiation in response to geographical distance and forest area

Overall differentiation among samples was relatively high with $F_{ST} = 0.12$ ($P \leq 0.001$). Mantel tests showed a significant correlation between genetic distances and geographical distances when considering all forest fragments ($n = 21$, $r = 0.440$, $P = 0.005$; Fig. 4a). This pattern emerged primarily because of the small genetic distance between samples originating from nearby forests. When focusing only on the Kolenwoud fragments we observe little genetic differentiation between the samples within the fragments (open circles in Fig. 4b) as compared to samples from different (nearby) fragments (filled circles in Fig. 4b). This suggests that gene flow within the often fairly large fragments is significant, in contrast to gene flow between fragments that are separated by modest stretches of unsuitable habitat matrix. 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 samples within forest fragments (0.24%; $P = 0.05$). Most of the variations, however, was explained by the variance within samples (93.39%; $P < 0.001$).

The linear model approach in GESTE (Foll & Gaggiotti, 2006) did not support a model that included the geographical structuring of the fragments. Highest support was found for a simple regression model that only included the effect of fragment area (posterior probability = 0.837), which had a negative effect on fragment specific F_{ST} -values, indicating that genetic differentiation decreased with increasing forest size (Fig. 5). Hence, samples from smaller forest fragments were significantly more differentiated than those from larger forest fragments. Only a weak and not significant degree of spatial autocorrelation in fragment area was observed (Moran's $I = 0.07$; $P = 0.16$).

Discussion

This population genetic study on the forest specialist and wingless beetle *C. problematicus* shows that the genetic constitution of samples originating from different forest fragments are, in general, strongly differentiated. Our linear model approach revealed that overall forest size is the main factor influencing the genetic structure of *C. problematicus*, wherein samples from smaller forest fragments exhibited a considerably higher genetic differentiation compared to those from larger forest fragments. This is in strong accordance with population genetic theory wherein the intensity of genetic drift in a population is inversely

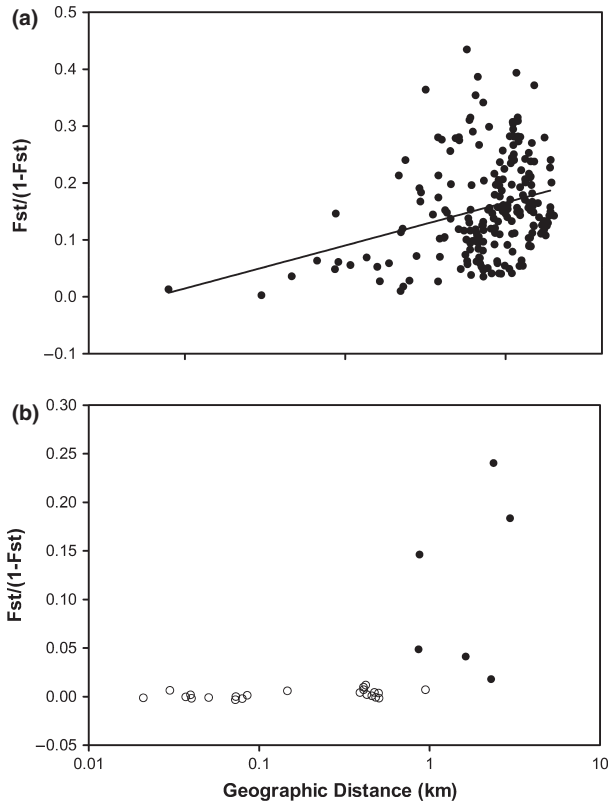


Fig. 4. Relationship between geographical (km) and genetic [$F_{ST}/(1 - F_{ST})$] distance for all sampled forest fragment populations of *Carabus problematicus* (a) and for Kolenwoud populations only (b). Open circles represent comparisons of populations from the same forest fragment filled circles represent population comparisons from different forest fragments.

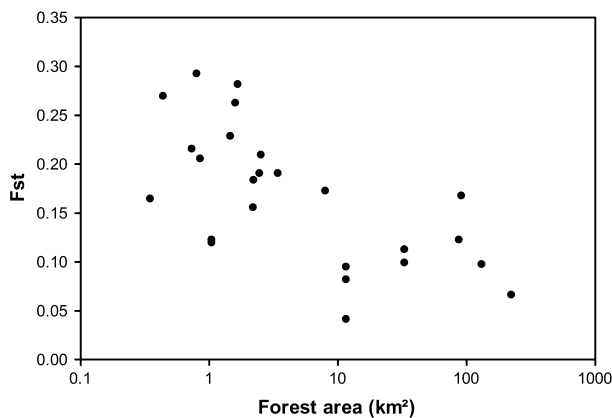


Fig. 5. Relationship between population specific F_{ST} -values and forest area in *Carabus problematicus*.

related to its effective size (Taylor, 2003; Hedrick, 2005). 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 local genetic diversity (Slatkin, 1987) and can further lead to decreased heterozygosity and inbreeding depression, which further enhances the risk of local extinction (Saccheri *et al.*, 1998; Frankham *et al.*, 2002). That this loss of local genetic diversity is currently ongoing in our smaller fragments was evidenced by a positive relationship between all measures of genetic diversity and forest fragment area. Although higher differentiation among populations may increase genetic variation at a regional level (Slatkin, 1987; Hanski & Gilpin, 1991), this pattern was not observed in this study. For example, unique alleles were almost only found in the larger forest fragments. A comparable result was found for other ground beetles, albeit in other habitat types (Desender, 2005; Drees *et al.*, 2011) and for other insects (Knutsen *et al.*, 2000; Williams *et al.*, 2003; Keyghobadi *et al.*, 2005) Whether or not this loss of genetic diversity effectively decreases the adaptive potential of these smaller populations remains, however, elusive as the use of neutral genetic markers only gives an indirect estimate of the genetic variation at loci under selection.

In Belgium, *C. problematicus* was never recorded in forests smaller than 30 hectares. Recent studies, however, suggested that observed area effects are driven by edge effects (Ewers *et al.*, 2007), as smaller fragments undergo proportionally stronger edge effects as compared to larger fragments (Murcia, 1995; Didham *et al.*, 1998a,b; Ewers & Didham, 2006; Ewers *et al.*, 2007; Fletcher *et al.*, 2007). This can result in a lower habitat quality of the smaller fragments and eventually to a lower genetic diversity. Disappearance of forest specialist species in fragmented patches is 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 & Niëmela, 1993; Lövei & Sunderland, 1996; Ås, 1999; Magura *et al.*, 2001, 2010; Summerville & Crist, 2004; Didham *et al.*, 2007; Ewers *et al.*, 2007; Hendrickx *et al.*, 2009). Ewers *et al.* (2007) found evidence of a strong interaction between habitat area and edge effects, changing exponentially with increasing fragment area. 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).

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 geographical scale of Belgium, the clear isolation-by-distance relationship at first suggests an equilibrium between drift and gene flow (Hutchison & Templeton, 1999). Yet, it should be noted that a smaller genetic distance among nearby samples remains hard to distinguish from a shared evolutionary history experienced by neighbouring samples. A more detailed visual inspection on a spatially more restricted dataset of the Kolenwoud samples, sharing the

same evolutionary history showed that 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. This suggests that the relationship between geographical and genetic distance was primarily caused by the small genetic distance of a few neighbouring samples, pointing towards migration events being restricted to those fragments. This result was confirmed with the AMOVA analysis where a higher variance was found among the different forest fragments compared to the samples within the same forest fragment, even at relatively large geographical distances.

The restricted dispersal of *C. problematicus*, is most probably not simply due to its absence of flight capability (Neumann, 1971; Rijnsdorp, 1980), but also largely based on its stringent habitat requirements. Indeed, in a former study conducted in the same area of Brussels, strict forest specialists were never found outside forest fragments, independent of the type of matrix and these species even avoided edge habitats (Gaublomme *et al.*, 2008). Brouat *et al.* (2003) showed that non-forested areas were partial barriers to gene flow for the forest specialist species *C. punctatoauratus* and the forest species *C. nemoralis*.

The results of our 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. Based on the genetic results obtained from this study and the fact that the species has not been observed outside the forest patches (Gaublomme *et al.*, 2008), it is unlikely that the high frequency of empty patches are caused by metapopulation dynamics occurring within this set of populations, wherein local extinctions are compensated by recurrent recolonisations (Hanski, 1998). Insights into the genetic structure of threatened populations, particularly those existing in fragmented habitats, are thus 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 specificity. Establishing new forests would therefore be of only limited use for specialist species, as they may not colonise new patches readily. A more suitable management option, derived from our study results, might be to preserve and enlarge existing forest fragments, while corridors connecting remaining habitat fragments would only be useful if they were substantial in area and would contain core old growth habitat.

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