Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles

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Although habitat fragmentation is suspected to jeopardize the long-term survival of many species, few data are available on its impact on the genetic variability of invertebrates. We assess the genetic population structure of the flightless ground beetle *Carabus violaceus* L., 1758 in a Swiss forest, which is divided into several fragments by a highway and two main roads. Eight samples were collected from different forest fragments and analysed at six microsatellite loci. The largest genetic differentiation was observed between samples separated by roads and in particular by the highway. The number of roads between sites explained 44% of the variance in pairwise F_{ST} estimates, whereas the age of the road and the geographical distance between locations were not significant factors. Furthermore, a comparison of allelic richness showed that the genetic variability in a small forest fragment isolated by the highway was significantly lower than in the rest of the study area. These findings strongly support the hypothesis that large roads are absolute barriers to gene flow in *C. violaceus*, which may lead to a loss of genetic variability in fragmented populations.

Keywords: ground beetles; *Carabus violaceus*; habitat fragmentation; roads; genetic differentiation; microsatellite

1. INTRODUCTION

During the last century, the intensity of land use has increased dramatically owing to the human population growth triggered by the industrial revolution 150 years ago. As a consequence, areas unaffected by human activities have become rare and the remaining habitats of many wild species are fragmented. Small patches surrounded by inhospitable areas may not be able to sustain their original flora and fauna owing to, for example, changes in microclimate, increased presence of predators or invasion of new species (Primack 1993). Furthermore, the remaining populations may be reduced in size, which increases the risk of inbreeding and accelerates the loss of genetic variability through random genetic drift (Hedrick 2000). Although the relevance of these genetic factors to the long-term survival of populations is not undisputed (Lande 1988), several studies indicate that they should not be neglected (Mills & Smouse 1994; Frankham 1996; Newman & Pilson 1997; Saccheri *et al.* 1998). Additionally, habitat fragmentation may increase the probability of local extinctions by destroying effective metapopulation structures (Gonzalez *et al.* 1998).

Habitat fragmentation caused by transport infrastructure has gained importance during the last couple of decades. In the USA, for example, the density of public roads is *ca.* 0.66 km km^{-2} (Forman 2000). In the densely populated area of Switzerland, this density is even higher at an average of 2.7 km km^{-2} (Oggier *et al.* 2001). For many species, roads are barriers to dispersal owing to physical obstacles such as fences, mortality caused by the collision with vehicles or deterrence by the unknown artificial habitat (Mader 1984; Oggier *et al.* 2001). The microclimate along a road, for example, can differ significantly from the conditions in the adjacent natural habitat (Mader 1979).

The impact of roads on the movement of individuals or on the genetic structuring of populations has been assessed in several recent studies. Most of this work has focused on vertebrates such as birds (Develey & Stouffer 2001), amphibians (deMaynadier & Hunter 2000; Carr & Fahrig 2001) or mammals (Clarke *et al.* 1998; Gerlach & Musolf 2000; Wang & Schreiber 2001). For invertebrates, however, very few results are available, for example, on snails (Baur & Baur 1990), grasshoppers (Rietze & Reck 1991) and ground beetles (Mader 1984), in spite of the prevalence and the importance of these organisms in all ecosystems (Wilson 1992). These studies indicate that roads may be very strong barriers to dispersal for invertebrates and could potentially have a dramatic influence on the genetic variability of these organisms.

Many ground beetles (Coleoptera, Carabidae), particularly the larger species, are unable to fly (Wachmann *et al.* 1995) but are quite mobile on the ground and able to cover distances of more than 10 m in 24 h and a few hundred metres in longer time-spans (Thiele 1977). None the less, they seem to be very reluctant to cross paved roads. In an extensive mark–recapture study on *Abax parallelepipedus* Piller & Mitterpacher, 1783, only one out of 742 recaptured individuals was found to have crossed a 6 m wide road (Mader 1984). In our study on *Carabus violaceus* L., 1758, we assessed whether this isolation was strong enough to lead to detectable genetic differentiation between populations on opposite sides of major roads within a relatively short period of time.

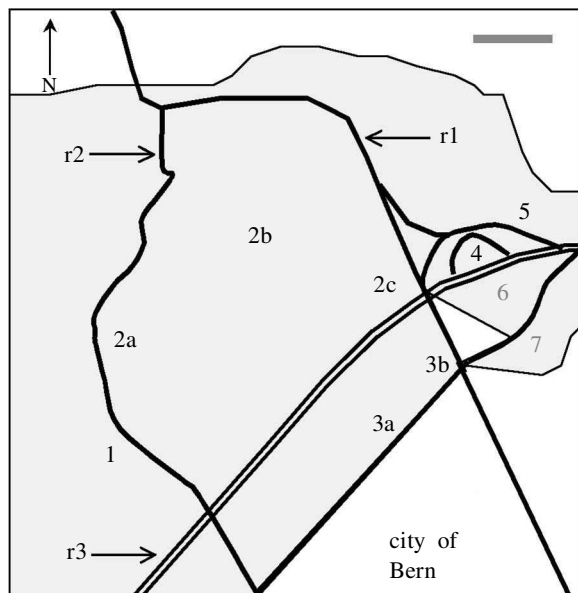*Author for correspondence (irene.keller@zos.unibe.ch).

Figure 1. Location of the sampling sites at Bremgartenwald, Bern. r1, main road (width 9 m; age minimum 130 years); r2, main road (width 8 m; age 87 years); r3, highway (width 30 m; age 31 years). Scale bar, 250 m.

Assuming a homogeneous habitat, we hypothesize that (i) the distances between our sampling sites (maximum 1.4 km) would be too small to lead to significant genetic differentiation as a result of isolation by distance in *C. violaceus*, given the aforementioned dispersal capacity of large ground beetles. In the absence of barriers to dispersal, we would therefore expect to find a genetically homogeneous population. Consequently, we further hypothesize that (ii) any genetic population structuring should be correlated with the number and/or the age of the roads between two sampling sites. Further, we tested whether the genetic variability in samples from a small fragment isolated by the highway was significantly lower than in samples from larger forest patches.

2. MATERIAL AND METHODS

(a) *Study species*

Carabus violaceus L. is a nocturnal ground beetle occurring in a variety of habitats throughout most of Europe (Thiele 1977; Wachmann *et al.* 1995). Our study focused on the subspecies *C. v. violaceus*, which is found in all types of forest (Marggi 1992). The beetles are between 22 and 35 mm in length and are unable to fly (Wachmann *et al.* 1995). The animals hatch in late summer, hibernate as larvae and enter their first reproductive period in the following summer. In our study population, very few individuals seem to go through a second reproductive period (I. Keller, unpublished data), and a generation time of 1 year can be assumed.

(b) *Sample collection*

The samples were collected during summer 2000 at 10 locations in a mixed forest near Bern, Switzerland (46°57′ N, 7°26′ E; figure 1). At site 4, additional samples were collected in 2001 to increase the sample size. The study area is divided into different fragments by a highway and two main roads (their ages and widths are given in the legend of figure 1). In a docu-

ment from 1870, road r1 is described as an artificial road of considerable width, but it is not known when it was paved.

The samples were collected using 16 dry pitfall traps per site arranged in a 15 m × 15 m grid. The traps consisted of plastic cups with a diameter of 11.5 cm and were emptied twice a week. For tissue collection, the animals were anaesthetized with CO_2, and the tarsus of one middle leg was cut off and stored in 100% ethanol. On the same day, the beetles were released back into the forest. At the beginning of the study, four treated and three untreated animals were kept in the laboratory for one month. During this period, only one untreated animal died, which indicated that the amputation of tarsi did not increase the mortality, at least under laboratory conditions. This is likely to also be true in the field, as we regularly recaptured individuals with missing tarsi. Additionally, a sample of 20 individuals from Zonien Forest near Brussels, Belgium (50°50′ N, 4°20′ E), at *ca.* 600 km from Bern, was obtained to serve as a reference for differentiation at a larger geographical scale.

(c) *Microsatellite typing*

The tarsi were ground with a plastic pestle, and the DNA was extracted with a Chelex resin extraction protocol (Estoup *et al.* 1996). All individuals were typed for six polymorphic microsatellite loci. The loci used were CVI05136CMPG, CVI08071CMPG, CVI09106CMPG, CVI09194CMPG and CVI10036CMPG isolated from *C. violaceus* (Keller & Largiadèr 2002) and 828A developed from *Carabus solieri* Dejean, 1826 (Rasplus *et al.* 2001). For the first five loci, the genotyping was carried out as described in Keller & Largiadèr (2002). For 828A, we used the same basic protocol with a $MgCl_2$ concentration of 2 mM and the following PCR profile: an initial denaturation step of 5 min at 95 °C, five cycles consisting of 1 min at 95 °C, 30 s at 54 °C and 75 s at 72 °C, 30 cycles consisting of 30 s at 94 °C, 30 s at 54 °C and 75 s at 72 °C, followed by a final 30 min extension at 72 °C. The PCR products were separated on an automated DNA sequencer (model 4200; LI-COR).

(d) *Statistical analyses*
(i) *Variation within populations*

Unless noted otherwise, all statistical analyses were conducted using ARLEQUIN v. 2.000 (Schneider *et al.* 2000), available from http://lgb.unige.ch/arlequin. For each locus in each sample, we estimated allele frequencies and observed and expected heterozygosities. In order to detect significant departures from Hardy–Weinberg equilibrium, we applied the procedure described in Guo & Thompson (1992) using a test analogous to an extension of Fisher's exact test to the case of multiple alleles. The tests were carried out with 1 000 000 steps in the Markov chain and 5000 dememorization steps. A sequential Bonferroni correction for multiple tests (Rice 1989) was applied to each population sample separately. For each locus in each sample, F_{IS} was calculated and tested for significant deviation from zero using FSTAT (Goudet 2001), available from http://www.unil.ch/izea/softwares/fstat.html. In each sample, we tested for linkage disequilibria between all pairs of the six loci by using a likelihood-ratio test (Slatkin & Excoffier 1996). We used 5000 permutations and 100 initial conditions for the EM algorithm in the permutation procedure implemented to obtain the null distribution of the likelihood-ratio statistic.

(ii) *Population differentiation*

Tests for significant genetic differentiation between samples were conducted using FSTAT. This program uses an exact *G-*

test, which is more powerful than other comparable tests, especially when the sample sizes are unequal (Goudet *et al.* 1996; Balloux & Lugon-Moulin 2002). The tests were carried out with the assumption of Hardy–Weinberg equilibrium within the samples using 1000 permutations.

Pairwise multilocus F_{ST} estimates were calculated using FSTAT based on the approach of Weir & Cockerham (1984). We did not calculate the R_{ST} estimates (Slatkin 1995), because their high associated variance may lead to unreliable estimates when the number of loci is small (Balloux & Lugon-Moulin 2002). Furthermore, some of our loci do not seem to follow a strict stepwise mutation model, in which case R_{ST} has been shown to seriously underestimate differentiation (Balloux *et al.* 2000).

A hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) implemented in ARLEQUIN was used to partition the total variance into covariance components at three different hierarchical levels: variance between groups, variance between samples within groups and variance within samples. For this analysis, the Belgian sample was omitted, and the Swiss samples were divided into five groups representing the five different forest fragments. We used 16 000 permutations to test the significance of the covariance components and fixation indices.

(iii) *Mantel test*

To assess the impacts of geographical distance and roads on the amount of genetic differentiation between samples, a Mantel test was carried out using FSTAT. By means of a permutation procedure, this analysis tested the significance of the partial regressions between the pairwise F_{ST} values and the following three matrices: geographical distance as measured on a map; number of roads; and minimum age of roads in years. We chose to use number and not width of roads because the historical information on the number of roads was more reliable. Two different weighting schemes were used, with the highway counted as one and two roads, respectively. The latter scheme was adopted because the highway is a dual carriageway (with a narrow grass strip in the middle) of more than twice the width of main roads. This weighting scheme best accounts for both potential barrier effects of roads (i.e. road width and number of roads). First, road width is likely to be correlated with mortality during crossings. Second, we expect the probability that an animal attempts to cross a road to be independent of road width, as the beetles probably cannot assess the width of a road from its edge. In this case, a highway with a grass strip in the middle may not be equivalent to a non-subdivided road of equal total width, because the animals are faced with two 'decisions' to cross. The determination coefficients were calculated as described in the treatment of path analysis in Sokal & Rohlf (1995).

(iv) *Reduction of genetic variability*

To detect a potential reduction in genetic variability in the samples from the small peripheral fragment south of the highway, we used FSTAT to calculate allelic richness as a measure of the number of alleles that is independent of the sample size (El Mousadik & Petit 1996). The values from samples 3a and 3b were compared with those from the remaining samples by means of an ANOVA implemented in JMP In 4.0 (SAS Inc.) with the six loci as repeated measures.

3. RESULTS

(a) *Sample collection*

We were able to obtain tissue samples from eight locations at Bremgartenwald. At sites 6 and 7 (figure 1), no *C. violaceus* were captured during the whole study period. The smallest sample was from site 4, where we caught only 16 different individuals in 2 years.

(b) *Genetic diversity and linkage*

All six microsatellite loci were polymorphic in all analysed samples, with the total number of alleles ranging between six and 12. The observed heterozygosities varied from 0.200 to 0.931, and only one out of the 54 values was significantly different from that expected under Hardy–Weinberg equilibrium (table 1).

Out of the 135 individual tests for linkage equilibrium, ten were significant at the 0.05 level and only two were significant at the 0.01 level. As these significant tests involved different pairs of loci in different samples, we concluded that they were likely to be the result of type I errors (a small number of tests are expected to be significant by chance) or within-population structuring (e.g. the result of finite population sizes) and not of physical linkage between loci. In the case of physical change, we would expect to find significant linkage between the same pairs of loci in several samples.

(c) *Population differentiation*

Out of the 36 pairwise tests for genetic differentiation, 28 (78%) were significant at the $p < 0.01$ level and 23 (64%) at the $p < 0.001$ level (table 2). Between pairs of samples from locations within the same forest fragment, two out of the four tests were significant at $p < 0.01$. Similarly, 58% ($n = 12$) of the pairwise comparisons between samples from opposite sides of a main road were significant at $p < 0.01$. Notably, almost all comparisons (92%; $n = 12$) between samples from opposite sides of the highway were significant at $p < 0.01$.

As expected, large F_{ST} values of around 0.4 were observed between the Belgian and the Swiss samples (table 2). Within the Swiss study area, we observed up to moderate differentiation with a maximum F_{ST} estimate of 0.057.

AMOVA revealed that most of the variance (97.4%) was explained by within-sample variation (table 3). Out of the remaining variance, slightly more was explained by differences between fragments than by differences between samples within the same fragment.

(d) *Mantel test*

The analysis with the highway counted as two roads revealed no significant partial regressions between pairwise F_{ST} values and the geographical distance or age of roads (table 4). The partial regression between the F_{ST} matrix and the number of roads, on the other hand, was highly significant ($p < 0.01$), with the variance in the number of roads explaining 44% of the variance in pairwise F_{ST} estimates. The same analysis with the highway counted as one road gave concordant results, but a less-pronounced partial regression between the F_{ST} matrix and the number of roads (partial regression coefficient = 0.0085; $p = 0.049$; standard partial regression coefficient = 0.529; 42% of the variance

Table 1. Genetic variability at six microsatellite loci in nine *C. violaceus* sampling areas.

(Listed are the number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, F_{IS} and the number of individuals scored in each sample (n). For each locus, repeat motive and GenBank Accession numbers are indicated.)

		locus name, repeat motif, accession number						
sample		828A (TG)$_n$(AG)$_n$	CVI05136 CMPG (TACA)$_n$ AJ436994	CVI08071 CMPG (CA)$_n$ AJ436995	CVI09106 CMPG (CA)$_n$ AJ436997	CVI09194 CMPG (CA)$_n$ AJ436998	CVI10036 CMPG (TG)$_n$ AJ436999	all
1	N_A	9	5	4	7	3	5	*5.5*
$n = 21$	H_O	0.857	0.667	0.333	0.714	0.619	0.810	*0.667*
	H_E	0.904	0.702	0.338	0.805	0.515	0.731	*0.666*
	F_{IS}	0.020	0.018	−0.112	0.138	−0.228	−0.079	*−0.013*
2a	N_A	9	6	3	8	4	5	*5.8*
$n = 29$	H_O	0.931	0.586	0.379	0.897	0.483	0.793	*0.678*
	H_E	0.840	0.742	0.426	0.833	0.491	0.707	*0.673*
	F_{IS}	−0.110	0.193	0.029	−0.081	−0.027	−0.134	*−0.028*
2b	N_A	7	6	5	9	4	5	*6.0*
$n = 22$	H_O	0.818	0.773	0.273	0.818	0.455	0.636	*0.629*
	H_E	0.831	0.806	0.362	0.781	0.461	0.691	*0.655*
	F_{IS}	−0.029	0.033	0.136	−0.091	−0.038	0.107	*0.008*
2c	N_A	7	6	5	9	7	5	*6.5*
$n = 35$	H_O	0.800	0.800	0.457	0.800	0.657	0.657	*0.695*
	H_E	0.786	0.808	0.467	0.817	0.651	0.668	*0.700*
	F_{IS}	−0.027	0.010	−0.024	0.009	−0.024	0.016	*−0.005*
3a	N_A	6	6	4	7	3	4	*5.0*
$n = 21$	H_O	0.906	0.619	0.571	0.762	0.429	0.476	*0.627*
	H_E	0.761	0.647	0.566	0.836	0.563	0.614	*0.665*
	F_{IS}	−0.098	0.060	−0.069	0.122	0.162	0.254	*0.069*
3b	N_A	5	7	4	8	4	3	*5.2*
$n = 46$	H_O	0.761	0.696	0.457	0.652	0.565	0.478	*0.602*
	H_E	0.736	0.717	0.531	0.735	0.561	0.588	*0.645*
	F_{IS}	−0.053	0.014	0.121	0.129	0.023	0.191	*0.065*
4	N_A	6	6	3	6	4	5	*5.0*
$n = 15$	H_O	0.733	0.733	0.200	0.800	0.667	0.800	*0.656*
	H_E	0.782	0.690	0.251	0.802	0.616	0.768	*0.652*
	F_{IS}	0.003	−0.050	−0.047	0.003	−0.056	0.000	*−0.019*
5	N_A	6	5	4	8	4	4	*5.2*
$n = 24$	H_O	0.750	0.750	0.458	0.542[a]	0.292	0.792	*0.597*
	H_E	0.791	0.725	0.537	0.798	0.368	0.726	*0.658*
	F_{IS}	0.095	−0.066	0.103	0.383[b]	0.155	−0.077	*0.102[c]*
Belgium	N_A	4	2	5	5	9	2	*4.5*
$n = 13$	H_O	0.615	0.231	0.692	0.308	0.769	0.154	*0.462*
	H_E	0.640	0.397	0.548	0.289	0.822	0.218	*0.486*
	F_{IS}	0.045	0.324	−0.279	−0.079	0.041	−0.032	*−0.009*

[a] Significantly different from H_E ($p = 0.007$).
[b] Significant deficit of heterozygotes ($p = 0.0019$).
[c] Over all loci: significant deficit of heterozygotes ($p = 0.0167$).

in pairwise F_{ST} estimates explained by the model). These results indicate that in *C. violaceus* no isolation by distance is observed at such a small scale. Furthermore, the number of roads between two locations has a much higher impact on genetic differentiation than does their minimum age.

(e) *Reduction of genetic variability*

The allelic richness in the two samples (3a and 3b) from the fragment south of the highway was significantly lower than that observed in the remaining samples (repeated measures ANOVA; $p = 0.014$). We detected a significant difference in allelic richness between loci ($p = 0.039$). A non-significant interaction between fragments and loci ($p = 0.587$) indicated that the allelic richness was consist-

ently higher in the large fragments to the north of the highway.

4. DISCUSSION

(a) *Population differentiation*

The data on genetic differentiation provided good support for the two hypotheses stated in § 1 as follows.

(i) *Hypothesis A*

An absence of isolation by distance (i.e. a genetically rather homogeneous population in the absence of artificial barriers) was expected based on the relatively high mobility of many ground beetles (Thiele 1977). The

Table 2. Pairwise F_{ST} estimates (below the diagonal) and p values of G-tests for pairwise differentiation (above the diagonal).
(Abbreviations: n.s., non-significant; Be, sample from Belgium.)

	Swiss samples								
	1	2a	2b	2c	3a	3b	4	5	Be
1	—	***	**	***	***	***	***	***	***
2a	0.0065	—	n.s.	**	n.s.	***	*	*	***
2b	0.0225	0.0045	—	*	**	***	n.s	n.s.	***
2c	0.0310	0.0122	0.0084	—	**	***	**	***	***
3a	0.0251	0.0077	0.0245	0.0193	—	***	***	***	***
3b	0.0461	0.0194	0.0391	0.0189	0.0211	—	***	***	***
4	0.0492	0.0186	0.0122	0.0170	0.0573	0.0447	—	n.s.	***
5	0.0387	0.0077	0.0059	0.0175	0.0428	0.0309	−0.0027	—	***
Be	0.4160	0.3860	0.4057	0.3707	0.4038	0.3977	0.4086	0.3954	—

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3. Genetic variance components and hierarchical F statistics for *C. violaceus* subpopulations from Bern.
(The subscripts of F refer to the hierarchical levels that are compared (FT, fragment to total population; SF, sample to fragment; ST, sample to total population.)

variance components	
between fragments	1.69%
between samples within fragments	0.91%
within samples	97.40%

F statistics	
F_{FT}	0.017**
F_{SF}	0.009***
F_{ST}	0.026***

** $p < 0.001$; *** $p < 0.0001$.

results of the Mantel test indicated that there was indeed hardly any correlation between genetic differentiation and geographical distance (table 4).

(ii) *Hypothesis B*

As we would expect if genetic differentiation is predominantly caused by artificial obstacles to dispersal, we observed a large number of significant F_{ST} values between samples from opposite sides of major roads. However, in the case of the main roads (r1 and r2) only 58% of the 12 pairwise comparisons between samples from different sides were significant at $p < 0.01$, which is less than expected. Some of the non-significant values could be caused by a lack of power owing to the very small size of sample 4 (16 individuals). Still, it is likely that, although the isolation caused by roads r1 and r2 may be somewhat underestimated, these main roads are not absolute barriers to gene flow. The highway, however, seems to have a very strong effect on population structure, and only one pairwise F_{ST} value between two samples from opposite sides was not significant at $p < 0.01$.

One of the two significant tests for differentiation between samples from the same fragment (sites 3a and 3b; table 2) may also be explained by a road effect; the two sites had been separated by a road until the late 1960s.

This so-called Glasbrunnenstrasse had been of considerable importance in the twentieth century, and had been used as a track for car races. The second of two significant tests for differentiation values between samples from the same fragment, which was observed between sites 2a and 2c (table 2), may be explained by a demographic effect. Data on activity density show that the abundance of *C. violaceus* is very low in some areas between these two sites (I. Keller, unpublished data), which could increase their isolation.

The strong impact of the roads was further supported by the results of the AMOVA (table 3) and particularly by the results of the Mantel test (table 4). The latter showed that 44% of the variance in the pairwise F_{ST} values could be explained by differences in the number of roads between sites, whereas the age of the roads was irrelevant. This is a further indication of the very strong impact of the highway, which, in spite of its quite recent construction, has led to levels of genetic differentiation between samples from opposite sides that are similar to those caused by the much older main roads. This may be explained either by an increased barrier effect of the highway or by increased genetic drift in the relatively small fragment (sites 3a and 3b) south of the highway.

It is improbable that the observed correlation between roads and genetic differentiation is an artefact and that the structuring of the beetle populations was caused by entirely different factors. In the study area, the stretches of the roads lying directly between sampling locations do not follow any conspicuous topographical elements. Therefore, we can assume that the construction of the roads within the study area was completely random with respect to natural factors relevant to population differentiation in *C. violaceus* and may even be regarded as an unplanned experiment.

The genetic data are in good agreement with information available from field studies. Mader (1984) showed that a 6 m wide road was a significant if not absolute barrier for the ground beetle *Abax parallelepipedus*, being crossed by only one out of several hundred individuals. Even if the exchange of effective migrants is rare, it may suffice to slow down or prevent genetic differentiation between the subpopulations separated by the road (Mills & Allendorf 1996). The road studied by Mader

Table 4. Results of the Mantel test: partial regression coefficients ($b_{Yj\cdot}$) between F_{ST} values and geographical distance, number of roads and age of roads, respectively, p values for $b_{Yj\cdot}$, standard partial regression coefficients ($b'_{Yj\cdot}$) and determination coefficients.

	$b_{Yj\cdot}$	p value	$b'_{Yj\cdot}$	determination (%)
geographical distance	7×10^{-6}	0.53	0.131	3.3
number of roads	0.009	0.009	0.623	44.0
age of roads	3.8×10^{-5}	0.67	0.109	5.8
% of variance explained by the model				53.1

(1984) may be comparable with our main roads r1 and r2, which we also suspect to be strong but probably not absolute barriers to gene flow (see above). The strong impact of the highway is supported by a field study by Zangger (1995), in which no forest ground beetle species were caught in pitfall traps installed for four weeks along the grass strip separating the two lanes of a highway.

(b) Loss of genetic variability resulting from habitat fragmentation

The isolation of ground beetle populations caused by the main roads and the highway in particular appears to be considerable. A comparison of allelic richness showed that the genetic variability in the small peripheral fragment south of the highway was significantly lower than that in the rest of the study area. It is improbable that this was the result of an edge effect, because no isolation by distance was observed in the study area (table 4) and a homogeneous population can be assumed in the absence of barriers to dispersal. In order to assess the importance and velocity of the loss of genetic variability, an estimate of the effective population size (N_e) in the small fragment (*ca.* 27 ha) is needed. This was obtained by assuming that the average heterozygosity in samples 2a, 2b and 2c from the large northern fragment represented the original value H_O before the construction of the highway. The heterozygosity in the southern fragment (samples 3a and 3b), on the other hand, was regarded as the value H_T after 30 generations of drift in a population of size N_e. Thus, we obtained average values of $H_O = 0.667$ (range of 0.629–0.695) and $H_T = 0.614$ (range of 0.601–0.627), and by applying $H_T = H_O \exp(-T/2N_e)$ (Hedrick 2000) estimated $N_e = 181$. It is clear that this analysis cannot provide more than a very rough estimate of N_e, but it seems reasonable to assume an effective population size of a few to several hundred individuals in the southern fragment. In the extreme case of a true N_e of *ca.* 100 animals, it would take 139 generations of complete isolation for the heterozygosity to be reduced to half of its original value (Hedrick 2000). This indicates that a habitat will have to be quite small (e.g. a few hectares) for genetic factors to become important in the long-term survival of *C. violaceus*, because the species occurs at relatively high densities (Amos & Balmford 2001). However, very small forest habitats are quite common in many densely populated areas. Our field data show that habitat fragmentation may indeed lead to local extinctions of *C. violaceus* in such small fragments, as the species is absent from patches 6 (*ca.* 2 ha) and 7 (*ca.* 5.55 ha) (figure 1). Although the situation before the highway construction is unknown, it is likely that this observation can be ascribed to the negative

effects of fragmentation, because *C. violaceus* occurs at high densities at location 5, which was connected to 6 and 7 until 30 years ago. However, such local extinctions are not necessarily the result of genetic factors (loss of genetic variability, inbreeding), but could also be caused, for example, by random demographic processes, because fluctuations in population size, which are very common in insects (Thomas 1990), cannot be buffered by recolonization (den Boer 1990).

(c) Conclusions

In conclusion, our findings strongly indicate that the isolation caused by major roads has a significant impact on the genetic structure of ground beetle populations. Furthermore, our results indicate that habitat fragmentation can lead to a loss of genetic variability and possibly to the extinction of local populations of *C. violaceus*. Thus, invertebrates should not be neglected in studies on fragmentation, and future efforts to improve the connectivity of habitats should consider the needs of these animals, for example by providing suitable microhabitats on wildlife overpasses (Zangger 1995).

REFERENCES

Amos, W. & Balmford, A. 2001 When does conservation genetics matter? *Heredity* **87**, 257–265.

Balloux, F. & Lugon-Moulin, N. 2002 The estimation of population differentiation with microsatellite markers. *Mol. Ecol.* **11**, 155–165.

Balloux, F., Brünner, H., Lugon-Moulin, N., Hausser, J. & Goudet, J. 2000 Microsatellites can be misleading: an empirical and simulation study. *Evolution* **54**, 1414–1422.

Baur, A. & Baur, B. 1990 Are roads barriers to dispersal in the land snail *Arianta arbustorum*? *Can. J. Zool.* **68**, 613–617.

Carr, L. W. & Fahrig, L. 2001 Effect of road traffic on two amphibian species of differing vagility. *Conserv. Biol.* **15**, 1071–1078.

Clarke, G. P., White, P. C. L. & Harris, S. 1998 Effects of roads on badger *Meles meles* populations in south-west England. *Biol. Conserv.* **86**, 117–124.

deMaynadier, P. G. & Hunter, M. L. 2000 Road effects on

amphibian movements in a forested landscape. *Nat. Areas J.* **20**, 56–65.

den Boer, P. J. 1990 The survival value of dispersal in terrestrial arthropods. *Biol. Conserv.* **54**, 175–192.

Develey, P. F. & Stouffer, P. C. 2001 Effects of roads on movements of understory birds in mixed-species flocks in Central Amazonian Brazil. *Conserv. Biol.* **15**, 1416–1422.

El Mousadik, A. & Petit, R. J. 1996 High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. *Theor. Appl. Genet.* **92**, 832–839.

Estoup, A., Largiadèr, C. R., Perrot, E. & Chourrout, D. 1996 Rapid one tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Mol. Mar. Biol. Biotechnol.* **5**, 295–298.

Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.

Forman, R. T. T. 2000 Estimate of the area affected ecologically by the road system in the United States. *Conserv. Biol.* **14**, 31–35.

Frankham, R. 1996 Relationship of genetic variation to population size in wildlife. *Conserv. Biol.* **10**, 1500–1508.

Gerlach, G. & Musolf, K. 2000 Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conserv. Biol.* **14**, 1066–1074.

Gonzalez, A., Lawton, J. H., Gilbert, F. S., Blackburn, T. M. & Evans-Freke, I. 1998 Metapopulation dynamics, abundance, and distribution in a microecosystem. *Science* **281**, 2045–2047.

Goudet, J. 2001 FSTAT, a program to estimate and test gene diversities and fixation indices (v. 2.9.3). See http://www.unil.ch/izea/softwares/fstat.html.

Goudet, J., Raymond, M., de Meeüs, T. & Rousset, F. 1996 Testing differentiation in diploid populations. *Genetics* **144**, 1933–1940.

Guo, S. W. & Thompson, E. 1992 Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.

Hedrick, P. W. 2000 *Genetics of populations*, 2nd edn. Sudbury, MA: Jones and Bartlett.

Keller, I. & Largiadèr, C. R. 2002 Identification of one X-linked and five autosomal microsatellite loci in *Carabus violaceus* (Coleoptera, Carabidae) and their applicability to related taxa. *Mol. Ecol. Notes* **2**, 290–292.

Lande, R. 1988 Genetics and demography in biological conservation. *Science* **241**, 1455–1460.

Mader, H. J. 1979 Die Isolationswirkung von Verkehrsstrassen auf Tierpopulationen untersucht am Beispiel von Arthropoden und Kleinsäugern der Waldbiozönose. *Schriftenreihe Landschaftspflege Naturschutz* **19**, 1–126.

Mader, H. J. 1984 Animal habitat isolation by roads and agricultural fields. *Biol. Conserv.* **29**, 81–96.

Marggi, W. A. 1992 *Faunistik der Sandlaufkäfer und Laufkäfer der Schweiz (Cicindelidae & Carabidae) Coleoptera*. Neuchâtel: Centre suisse de cartographie de la faune.

Mills, L. S. & Allendorf, F. W. 1996 The one-migrant-per-generation rule in conservation and management. *Conserv. Biol.* **10**, 1509–1518.

Mills, L. S. & Smouse, P. E. 1994 Demographic consequences of inbreeding in remnant populations. *Am. Nat.* **144**, 412–431.

Newman, D. & Pilson, D. 1997 Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution* **51**, 354–362.

Oggier, P., Righetti, P. & Bonnard, L. (eds) 2001 *Zerschneidung von Lebensräumen durch Verkehrsinfrastrukturen COST 341. Schriftenreihe Umwelt*. Bern: Bundesamt für Umwelt, Wald und Landschaft; Bundesamt für Raumentwicklung; Bundesamt für Verkehr; Bundesamt für Strassen.

Primack, R. B. 1993 *Essentials of conservation biology*. Sunderland, MA: Sinauer.

Rasplus, J.-Y., Garnier, S., Meusnier, S., Piry, S., Mondor, G., Audiot, P. & Cornuet, J. M. 2001 Setting conservation priorities: the case study of *Carabus solieri* (Col. Carabidae). *Genet. Sel. Evol.* **33**, 141–175.

Rice, W. R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Rietze, J. & Reck, H. 1991 Untersuchungen zur Besiedlung der Verkehrsnebenflächen des Autobahnkreuzes Stuttgart durch Heuschrecken (Orthoptera, Saltatoria) mit besonderer Berücksichtigung der Dispersion der Grossen Goldschrecke (*Chrysochraon dispar*). *Articulata* **6**, 91–119.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. 1998 Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494.

Schneider, S., Roessli, D. & Excoffier, L. 2000 ARLEQUIN v. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva.

Slatkin, M. 1995 A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 157–162.

Slatkin, M. & Excoffier, L. 1996 Testing for linkage disequilibrium in genotypic data using the EM algorithm. *Heredity* **76**, 377–383.

Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*, 3rd edn. New York: W. H. Freeman.

Thiele, H. U. 1977 *Carabid beetles in their environments*. Berlin: Springer.

Thomas, C. D. 1990 What do real population dynamics tell us about minimum viable population sizes? *Conserv. Biol.* **4**, 324–327.

Wachmann, E., Platen, R. & Barndt, D. 1995 *Laufkäfer-Beobachtung, Lebensweise*. Augsburg, Germany: Naturbuch.

Wang, M. & Schreiber, A. 2001 The impact of habitat fragmentation and social structure on the population genetics of roe deer (*Capreolus capreolus* L.) in Central Europe. *Heredity* **86**, 703–715.

Weir, B. S. & Cockerham, C. C. 1984 Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Wilson, E. O. 1992 *The diversity of life*. Cambridge, MA: The Belknap Press.

Zangger, A. 1995 *Wildlife overpasses over a motorway as connecting means for forest arthropod communities*. PhD thesis, University of Bern, Bern, Switzerland.