The effects of habitat fragmentation on demography and on the loss of genetic variation in the red squirrel

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SUMMARY

A major problem in conservation biology is the extent to which the loss of genetic variability in isolated populations reduces their chance of survival. We present data in which the loss of genetic diversity in small and isolated populations can be directly related to population dynamics. Genetic similarity in red squirrels is inversely correlated with population size. The loss of genetic variation and the lower population densities in isolated populations are both the result of reduced immigration. Our data suggest that population processes rather than genetic problems are the real threat to small squirrel populations.

1. INTRODUCTION

Loss of genetic variation may be one of the reasons why small populations become extinct, and it has been suggested that the minimum effective population size to maintain 'sufficient' variation to ensure long-term survival must be at least 500 individuals (Franklin 1980). This conclusion is, however, based on models for which the following assumptions are made: (i) the loci used to measure genetic variation are selectively neutral and distributed as for quantitative traits in *Drosophila*, corn and mice (Lande & Barrowclough 1987); and (ii) viability affecting loci are distributed in populations as they are in *Drosophila*. For many species, these conditions will not be met. Moreover, it is known that the level of heterozygosity varies considerably among species (Nevo 1978). Therefore, estimates of minimum viable populations (MVPs) are often in the thousands (Soulé 1987).

Laboratory and captive breeding programmes provide extensive evidence of increased juvenile mortality with inbreeding (Ralls & Ballou 1982, 1986; De Bois *et al.* 1990). However, field evidence shows highly inbred wild populations that survive and apparently thrive despite the fact that most of their genetic variability has been lost (Bonnell & Selander 1974; Gilbert *et al.* 1990). Soulé (1987) has argued that the finding that 'some bottlenecks do not lead to immediate extinction' should not be generalised to bottlenecks do not lead to extinction. However, there are simply not enough data to evaluate the effects of isolation caused by habitat fragmentation, and the associated loss of genetic variation upon population survival. For this evaluation we need data on genetic variation and demographic parameters in a series of populations of the same species.

We present here such data from a comparative study of two large ('mainland') and five small ('island') populations of the Eurasian red squirrel, *Sciurus vulgaris* L., from a restricted area near Antwerp, Belgium.

2. METHODS

We have measured population parameters directly by using marked and radio-tagged individuals (table 1), and genetic variation by using DNA fingerprints derived from the 'Jeffreys' probe (table 2).

Red squirrels were studied during 4 years (1989–1992) in two large, open habitats ('mainland habitats'), during 2 years in one isolated woodland (1991–1992), and during 1 year in four isolated plots (two in 1991, and two in 1992) ('island habitats'). The results for 1989 and 1990 have been added to show that the two years 1991 and 1992, in which the comparisons were made between mainland and island populations, were not atypical. Area C (300 ha†) was a mixed coniferous forest, dominated by Scots pine, *Pinus sylvestris*, and Corsican pine, *Pinus nigra*. Area D (600 ha) was a mixed deciduous forest with mainly oak, *Quercus robur*, beech, *Fagus sylvatica*, and chestnut, *Castanea sativa*, and a few Scots pine stands. In both large forests, study plots of 30 ha were used (Wauters & Dhondt 1990). Areas X (35 ha, study plot of 25 ha), and K (36 ha) were isolated woodlands similar in tree composition to area D, and area I (60 ha, study plot of 33 ha) was a mixed woodland with 70% Scots and Corsican pine and 30% oak, beech and red oak, *Quercus rubra*. Area B consisted of many very small woodlots (total 38 ha) with mainly mature oak, beech and poplar, with some mixed pine stands. Area H was a partly forested (55 ha) moorland covered with young and mature Scots and Corsican pine and some oak, mixed with open areas unsuitable for squirrels.

Woodland homogeneity was calculated by using the

† 1 ha = 10^{-4} m^2.

Table 1. *Population data for island and mainland red squirrel populations*
(Summer adult density per hectare (adult), summer adult female density (FF), annual survival rate of residents, the mean number of young per adult female (young per FF), and the mean number of young weaned per successfully reproducing female per year (young per sFF), local juvenile survival rate (juvenile survival = number (*n*) and proportion (rate) of weaned young) and immigration (immigration = number (*n*) or number per adult per year (rate)). Mean adult body mass (in grams) of residents in February–March was calculated as a measure of the overall condition of squirrels in a population. Sample size for body mass, the number of adults weighed = (*n*).)

		density/ha^{-1}					immigration		juvenile survival		body mass	
plot	year	adult	FF	survival rate	young per FF	young per sFF	*n*	rate	*n*	rate	mean±s.d.	(*n*)
isolated plots: 1991–1992												
X	91	0.60	0.28	0.61	1.71	2.40	1	0.07	6	0.50	316±24	(15)
X	92	0.76	0.32	0.61	0.90	1.80	1	0.05	2	0.20	318±16	(16)
B	91	0.44	0.21	0.58	0.88	1.75	1	0.06	2	0.29	317±21	(16)
I	91	0.45	0.24	0.60	1.57	2.20	3	0.13	4	0.33	329±32	(13)
H	92	0.52	0.24	0.65	1.40	2.33	3	0.14	3	0.23	331±31	(17)
K	92	0.64	0.20	0.65	1.60	2.00	4	0.25	2	0.22	341±26	(15)
mainland plots: 1991–1992												
C	91	1.17	0.53	0.68	1.38	2.14	12	0.32	4	0.27	334±15	(22)
C	92	1.07	0.47	0.64	1.29	2.25	8	0.25	2	0.15	343±18	(20)
D	91	1.23	0.60	0.62	1.42	2.13	13	0.35	2	0.14	340±22	(30)
D	92	0.97	0.43	0.57	0.85	2.20	7	0.24	2	0.17	321±22	(25)
mainland plots: 1989–1990												
C	89	1.00	0.47	0.56	1.00	1.86	7	0.23	2	0.25	345±19	(20)
C	90	1.03	0.50	0.55	1.58	2.71	19	0.46	2	0.16	331±21	(24)
D	89	0.80	0.43	0.61	0.83	1.67	3	0.13	3	0.36	334±20	(22)
D	90	1.29	0.50	0.72	1.69	3.38	14	0.39	4	0.18	336±23	(23)

Table 2. *Genetic variation, obtained by using DNA fingerprints derived from 'Jeffreys' probes*
(Woodland diversity (1-*D*), trap-cover index (size of trapping grid/total woodland size), adult population size, estimated by multiplying adult summer density with total woodland size (see table 1), the mean number of clearly defined minisatelite fragments (bands, *n* = the number of squirrels for which bands could be counted), and the genetic similarity coefficient for each study site (*n* = number of adjacent tracks, squirrel-pairs, used to calculate the mean genetic similarity).)

				bands			similarity		
site	1-*D*	trap cover	population size	*n*	mean	s.d.	*n*	mean	s.d.
C	0.47	0.1	336	16	18.56	3.69	25	0.538	0.147
D	0.54	0.05	660	19	21.16	3.76	7	0.450	0.122
X	0.34	0.71	21	33	21.06	4.66	26	0.631	0.157
B	0.58	1.0	17	36	21.25	3.43	20	0.729	0.096
I	0.48	0.55	27	26	19.89	3.67	17	0.570	0.172
H	0.56	1.0	29	19	22.84	4.22	21	0.565	0.115
K	0.57	1.0	23	13	22.15	3.78	7	0.547	0.071

Simpson's index of diversity, 1-*D* (Krebs 1989): $1\text{-}D = 1-\Sigma p_i^2$, where p_i is the proportion of species i in the community. To calculate *D*, three species–age classes of trees were defined: (i) mature oak–beech; (ii) mature pine; and (iii) all other forest types (table 2).

Between January and November, populations were studied with capture–mark–recapture techniques, combined with radio-tracking (Wauters & Dhondt 1990, 1992). Intensive, continuous trapping from January to March in each study area allowed us to mark all squirrels on the study plots. Thus, by the end of March, before reproduction and the start of the main dispersal period (Wauters & Dhondt 1990, 1993), all residents in all populations were individually marked allowing exact measurements of the population parameters presented in table 1.

Density on the study plots was measured as the number of marked squirrels per hectare. If the study plot covered only part of the woodland (areas C, D, I and X), radio-tracked squirrels that had their home ranges off the study plot and squirrels trapped only in edge traps were excluded, so as not to overestimate density in these areas. Survival rate was measured for all squirrels present in March which were at least 8 months old. In all populations, these squirrels were considered as residents. Survival rate was calculated from the proportion of residents present in March which were still present at the end of November (*ms* = survival over 8 months) and extrapolated to an annual (12 months) survival rate (*sr*) with the formula: $sr = e^{12/8\,(\ln ms)}$.

All lactating females were marked with radio-collars and their nest sites located. In each study plot, all weaned juveniles were marked in the nest, trapped around the nest tree, or observed and counted. The mean number of weaned young per adult female and the mean number of weaned young per female that raised offspring (successful female)

were calculated to measure reproductive rate. Of the young born in March–April and weaned in May–June (spring litters), local survival was measured up to 8 months of age (November) by using recaptures, observations and radio-tracking of young.

All unmarked squirrels caught from April to November which survived at least 2 months on the study plots were considered immigrants. Thus only squirrels not present in winter (January–March) which immigrated and actually settled on the study plots, i.e. those recaptured at least once, 2 months or more after first capture, and those radio-tracked with home ranges on the study plot, were classified as immigrants, to avoid overestimating the number of immigrants at mainland sites and at sites I and X. Immigration rate was calculated as the number of immigrants per resident adult per year. Data on long-term immigration success showed that, in plots C and D, 61% of immigrant females and 78% of immigrant males actually reproduced (Wauters & Dhondt 1993).

DNA was prepared from ear tissue by homogenization in STE buffer (Wetton *et al.* 1992) followed by extraction using phenol/chloroform. Restriction was with Mbo-1, followed by electrophoresis through 1% agarose gels (24 cm × 20 cm) for 1850 V h^{-1}. Fragments were transferred to Zetaphrobe GT membrane by Southern blotting, and probed with the Jeffreys probe 33.6 subcloned into pSPT 19.6 (Carter *et al.* 1989). To reduce the problems of statistical independence, each individual was used only once in this analysis. The number of clearly defined minisatellite fragments was counted for each track (N), and the number of bands shared between adjacent tracks a and b (N_{ab}) recorded. Genetic similarity between two adult squirrels (a and b) from the same population was calculated by comparing each track (a) with the one to its right (b) on each blot using the formula: $2N_{ab}/(N_a + N_b)$.

The limited pedigree data support the conventionally accepted inheritance of hypervariable minisatellite (Jeffreys *et al.* 1985*a*, *b*).

To compare the demographic parameters between mainland and island populations in 1991 and 1992 (table 1), we used a two-way ANOVA (factor area = mainland or island; factor year = 1991 or 1992) with the assumption that points in different years in the same plot are independent.

3. RESULTS

The estimated size of the adult population varied from 17 to 29 squirrels in the fragments, and from 336 to 660 squirrels in the large woodlands (table 2). Also, population density in mainland plots (0.97–1.23 ha^{-1}) was higher than in fragments (0.44–0.76 ha^{-1}; table 1), (two-way ANOVA: area effect $F_{1,6} = 32.99$, $p < 0.001$; year effect $F_{1,6} = 0.33$, n.s.; area by year interaction $F_{1,6} = 1.44$, n.s.).

To test which demographic parameters were correlated with these differences in density, we compared reproductive rate, survival rate and immigration rate between mainland and island populations. Immigration rate (table 1) was higher in mainland plots (area effect $F_{1,6} = 18.46$, $p < 0.01$; year effect $F_{1,6} = 0.14$, n.s.; area by year interaction $F_{1,6} = 3.46$, n.s.), but local juvenile survival (table 1) was slightly higher in fragments (area effect $F_{1,6} = 5.35$, $p = 0.06$; year effect $F_{1,6} = 4.31$, n.s.; area by year interaction $F_{1,6} = 1.32$, n.s.). None of the other population measures adult survival rate and reproductive rate, nor adult body mass, differed significantly between mainland and island plots (figure 1).

Immigration rates could be overestimated in mainland population and in those island populations where the trapping grid (study plot) was smaller than the total woodland area ('trap-cover' effect). The trap-cover index (table 2), however, was not significantly correlated either with immigration rate ($r = -0.655$, $n = 7$, n.s.) or genetic similarity ($r = 0.648$; $n = 7$; n.s.).

Fragmented woodlands could be more homogeneous than large woodlands. Habitat diversity (table 2), however, did not differ between mainland and island plots (Mann-Whitney U test: $U = 3$, $n_1 = 2$, $n_2 = 5$, n.s.) and was not correlated with genetic similarity ($r = -0.069$, $n = 7$, n.s.).

Table 2 shows that the two large populations have, as expected, significantly lower band-sharing and similarity coefficients than the five smaller populations (Mann-Whitney U test: $U = 0$, $n_1 = 2$, $n_2 = 5$, $p = 0.026$, one-tailed). Genetic similarity increases significantly as population size decreases (correlation of similarity with log population size $r = -0.75$, $n = 7$, $p = 0.027$, one-tailed). However, there is also a negative correlation between band-sharing and immigration rate ($r = -0.857$, $n = 7$, $p < 0.01$; figure 2). A stepwise multiple regression analysis shows that, once the immigration rate has been entered in the model, population size does not explain additional variation, but not the converse (partial residual significance of log adult population size on genetic similarity, after immigration rate has been entered into the model, is $p = 0.79$). Thus immigration rate and not population size explains genetic similarity.

4. DISCUSSION

Studies of naturally fragmented populations have often been bedevilled by the lack of suitable genetic markers for a detailed analysis. The discovery of VNTR polymorphisms (Jeffreys *et al.* 1985*a*, *b*) offered a new suite of hypervariable loci that have been used for monitoring genetic changes among such populations. These DNA fingerprinting techniques detected reduced genetic variation in isolated or highly inbred populations (Gilbert *et al.* 1990, 1991; Reeve *et al.* 1990).

Small populations with reduced genetic variation are more likely to become extinct than large populations (Soulé 1987). The increased fragmentation of natural or semi-natural habitats in most of Europe during the last century has divided many once panmictic populations into different subpopulations that are more or less isolated from each other. Therefore the effects of habitat fragmentation on the survival of small populations has become a main issue in modern conservation biology (Soulé 1987). To evaluate the impact of increasing isolation caused by habitat fragmentation and its associated loss of genetic variation on population survival, we studied, simultaneously, genetic variation and demography in seven populations of the red squirrel, a species restricted to woodland habitats.

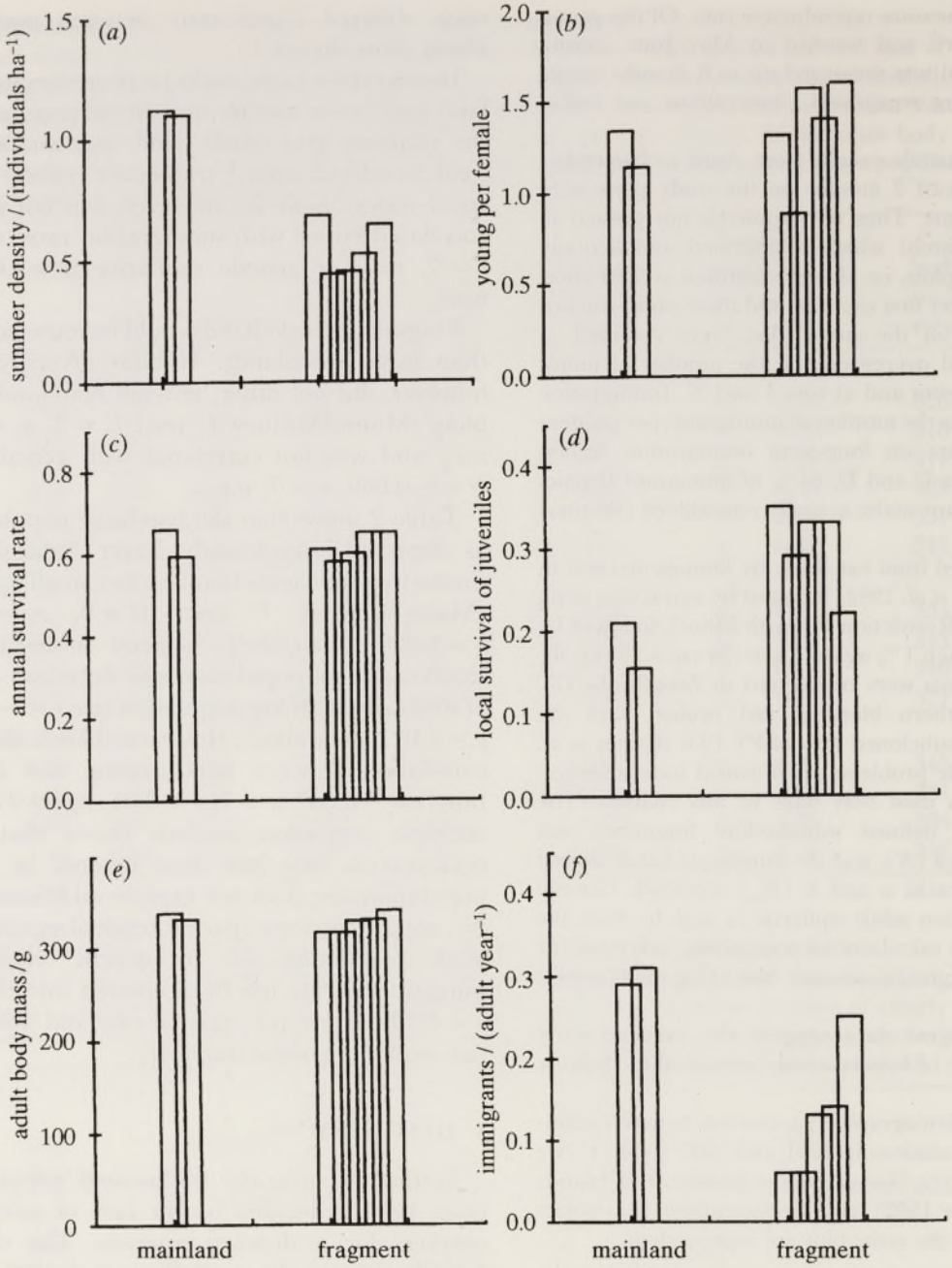

Figure 1. Demographic parameters (see table 1) in the two mainland areas and five woodland fragments. The sequence of the blocks is the same in each graph: mainland areas C, D, fragments X, B, I, H and K. (*a*) Summer density, (*b*) young per female, (*c*) annual survival rate, (*d*) local survival of juveniles, (*e*) adult body mass, and (*f*) immigration.

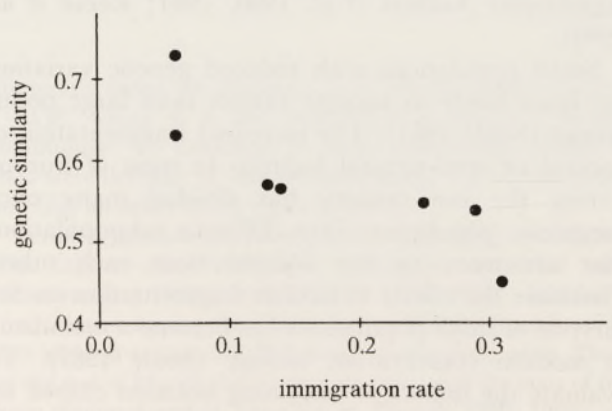

Figure 2. The correlation between immigration rate (number of immigrants per adult per year) and genetic similarity (expressed as the proportion of shared bands).

The interpretation of our results depends strongly on the accuracy of our measurement of immigration rate. In the mainland populations, C and D, which have been studied for many years, residents and immigrants could be distinguished easily (Wauters & Dhondt 1993) and immigration rate could be measured exactly by defining immigrants as squirrels that entered and established a home range on the study plot. In the fragmented populations, squirrels classified as residents could have immigrated and settled before winter (before January–March). However, also in mainland populations, immigrants that established a home range before January were regarded as residents the following year. Thus, by measuring immigration rate over a well-defined time period, April–November, the number of immigrants can be compared between mainland plots and fragments.

Higher adult squirrel densities in mainland populations were the effect of higher immigration rates in mainland plots than in fragments. A slightly higher local juvenile survival in the more isolated populations produced an insufficient increase in squirrel numbers to compensate for the decreased immigration rate. Population K had a lower local juvenile survival and a higher immigration rate than other island populations (figure 1). Dispersal to and from a nearby (smallest distance *ca.* 200 m) woodland fragment with a small squirrel population was observed (Wauters *et al.* 1994). The proximity of this small population explains the higher immigration rate in area K.

That members of a population with high immigration are genetically less similar to one another than those from a more isolated population with reduced immigration is not really surprising. What is important in this result is that, in a species that normally lives in extented habitats, and in which populations have only recently become fragmented, there seem to be no detectable negative effects of increased inbreeding on survival and reproduction, or on the condition (body mass) of the squirrels. What is also striking in our results is that the effects of demography have overcome the genetical consequences of finite populations, supporting the contention (Gilpin 1987) that ecological effects may be more significant than population genetics in determining the outcome of population fragmentation. Furthermore the fact that genetic similarity gradually decreases as immigration rate increases (figure 2), and that genetic variation is lost also when there is at least one immigrant per year (tables 1 and 2), shows that the accepted wisdom that a single successful immigrant per generation (Lande & Barrowclough 1987; Soulé 1987) would be sufficient to maintain genetic variation in a population is not supported by our data.

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