Absence of a genetic bottleneck in a wild rabbit (*Oryctolagus cuniculus*) population exposed to a severe viral epizootic

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Abstract

Infectious diseases and their demographic consequences are thought to influence the genetic diversity of populations. In Europe, during the last 50 years, the European rabbit (*Oryctolagus cuniculus*) has suffered two important viral epizootics: myxomatosis and rabbit viral haemorraghic disease (RVHD). Although mortality rates were very high, the impact of these diseases on genetic diversity has never been assessed directly. The subject of this paper is a wild rabbit population in France, which has been studied since the beginning of the 1980s. The first outbreak of RVHD occurred in 1995 and provoked a demographic crash. The population, sampled for the first time in 1982 and 1994, was sampled again at the end of 1996 to examine the impact of the epizootic on genetic diversity. In spite of the observed high mortality rate ($\approx 90\%$), analysis of 14 polymorphic loci (allozymes and microsatellites) showed no loss in genetic diversity after the epizootic. Determination of temporal changes in allele frequencies indicated that the population evolved under genetic drift. The temporal method of Waples demonstrated a significant decrease in the effective population size (N_e) correlated with the demographic crash due to the epizootic. However, the population had only been studied for two generations after the epizootic and the remnant population size probably stayed high enough (≈ 50 individuals) to keep its genetic diversity at the precrash level. These results suggest that, contrary to what is usually thought and in spite of the subsequent high mortality rates, past epizootics (especially myxomatosis) may have had little effect on the genetic diversity of wild rabbit populations in Europe.

Keywords: allozyme, bottleneck, European rabbit, microsatellite, viral haemorrhagic disease

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Introduction

Infectious diseases can have serious consequences for the demography and genetic diversity of populations (for a review, see O'Brien & Evermann 1988). When population size declines, theory predicts a loss of genetic variability (Wright 1931), inbreeding and fixation of deleterious alleles resulting in lowered disease resistance and decreased reproductive success (Ralls *et al.* 1979; Quattro & Vrijenhoek 1989; Charlesworth *et al.* 1993; Jiménez *et al.* 1994; Lande 1994; Frankham 1995). For example, very low allozyme diversity, monomorphism at the major histocompatibility complex, developmentally abnormal spermatozoa and increased juvenile mortality rates were observed in bottle-necked populations of lions and cheetahs (Wildt *et al.* 1987; O'Brien *et al.* 1983, 1985). However, in other species (*Rhinoceros unicornis*, *Perameles gunnii*, for example) bottlenecked populations retained relatively high levels of variability (Pimm *et al.* 1989; Robinson *et al.* 1993).

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Generalized predictions relative to the effects of diseases on genetic variability are very difficult to establish, because they bear on specific properties.

The European rabbit (*Oryctolagus cuniculus*) offers a good opportunity to investigate this problem. In Europe, rabbit populations have recently suffered two important viral diseases: myxomatosis at the beginning of the 1950s and rabbit viral haemorragic disease (RVHD) in the late 1980s. Myxomatosis has been the most important new wildlife disease in Europe this century, in particular in France and Britain (Fenner & Ross 1994). Before 1950, the myxoma virus was not present outside the American continent where its natural host is the cotton-tail rabbit (*Sylvilagus brasiliensis* and *S. bachmani*). Introduced into France in 1952, the disease had spread throughout the country by the end of 1953 and into most other European countries during 1954 and 1955. Several studies state a 99% mortality rate but this high value was an extrapolation of myxoma virus virulence based on the very high mortality rates of the initial outbreaks. A 90% value at the population level seems more realistic in the first year of epizootic (Fenner & Ross 1994; Rogers *et al*. 1994). Soon after the introduction of the myxoma virus, attenuated strains were recovered from naturally infected rabbits. Studies over the last 40 years show co-evolution between the virulence of the virus and resistance of the rabbit, although myxomatosis still exerts a substantial effect on the density of rabbit populations (Fenner & Ross 1994).

RVHD was first described in China in 1984 (Liu *et al*. 1984), reported in Europe in 1988 (Morisse *et al*. 1991; Mitro & Krauss 1993) and is now endemic in France (Barrat *et al*. 1996; Artois *et al*. 1997). Its impact on wild populations has only been reported by Villafuerte *et al*. (1995) in Spain, Marchandeau *et al*. (1998a) in France and Mutze *et al*. (1998) in Australia where RVHD was introduced to control rabbit populations. These studies show that during the first epizootic, mortality rates were generally high, 55–90%. As has been the case for myxomatosis, most European populations have developed anti-RVHD antibodies, which suggests that they are at least partially protected against this disease (Maess *et al*. 1991; Trout *et al*. 1997; Marchandeau *et al*. 1998b). A steady exposure to nonpathogenic RVHD-like viruses could be responsible for the high levels of immunity recorded in free-living populations (Marchandeau & Boucraut-Baralon 1999) as has already been shown in domestic rabbits (Capucci *et al*. 1996, 1997).

There is little information about the impact of the mortality induced by these epizootics on diversity and structure of rabbit populations. Van der Loo (1993) observed that genetic differentiation at the antibody loci was very low among wild populations from Continental Europe, Great Britain and Australia, in spite of putative founder effects and myxomatosis-related population bottlenecks, and suggests that this could be due to overdominant selection pressure observed at the studied loci (van der Loo & Verdoodt 1992). Surridge *et al*. (1999a) assert that a possible myxomatosis bottleneck could explain the high degree of genetic differentiation observed between populations within England. In other species, population bottlenecks are usually documented by direct counting or inferred from the subsequent genetic situations (O'Brien & Evermann 1988). However, the historical population sizes and level of genetic variation are rarely known precisely. In the absence of sampling before a demographic crash, temporal changes in allele frequencies can be assessed by an analysis of DNA extracted from ancient remains found in museums and/ or archeological sites (Taylor *et al*. 1994; Nielsen *et al*. 1997; Bouzat *et al*. 1998), but the available samples are rarely taken from the same population. Most of the time, comparisons are established with a related population or species (Bonnell & Selander 1974; Daley 1991; Packer *et al*. 1991; Taylor *et al*. 1994; Taylor & Cooper 1999).

The purpose of the present study was to examine a rabbit population for which genetic and ecological data from before the demographic crash were available. A first outbreak of RVHD occurred in 1995 in a wild population that had been monitored since the beginning of the 1980s. Annual mortality rates in 1995 were estimated at 88% in adults and 99% in juveniles (Marchandeau *et al*. 1998a). The population was sampled in 1982 and 1994 and other samples were taken in 1996, which allowed the study of the genetic consequences of the decrease in population size. We analysed the variation of mitochondrial DNA, allozyme and microsatellite loci to assess the effects of the epizootic on: (1) genetic diversity, (2) changes in allele frequencies, and (3) the effective size of the population.

Materials and methods

Sampling and demography

Since the beginning of the 1980s, ecological studies have been conducted on a wild population established in an arboretum (Chèvreloup) located at Versailles near Paris (France). The rabbit population was living on a 15-ha area where a subgroup of rabbits was monitored more intensively in a 5-ha central area (Fig. 1). From 1989 to 1997, rabbits were caught by trapping between January and September (the period corresponding to their breeding season) and using ferrets (*Mustela furo*) from October to December. All animals were marked with reflecting ear tags allowing individual recognition from a distance. Blood samples were taken during autumnal ferreting in 1982 ($n = 60$), 1994 ($n = 47$) and 1996 ($n = 51$).

Three methods of recapture (ferreting, trapping and spotlighting) were used to monitor population size (Marchandeau *et al*. 1998a). Ferreting on the 15-ha territory was performed for 4 weeks each year to physically capture all rabbits

Fig. 1 The 15-ha territory and the warrens (networks of burrows) where the rabbit population is established. The 5-ha central area is delineated by a grey line.

100 m

Trees and bushes
Empty warrens after epizootic
Occupied warrens after epizootic

living on that territory. The data obtained were used to determine the population size each year in January. In 1995, from January to September, for 5 days every 3 weeks, the numbers of living adults and juveniles were estimated on the 5-ha central area by spotlighting and trapping. This last method does not permit an evaluation of the total population size (i.e. on the 15-ha area) but it gives a reliable, monthly assessment of changes in population size. Because of the great heterogeneity of capture and the strong trap-response in rabbits, the enumeration method ('minimum number of known alive') used here, was preferable to probabilistic methods although it does not provide confidence intervals on estimated population sizes (Seber 1986; Pollock et al. 1990). A good estimate of the minimum number of known living rabbits was nevertheless obtained through numerous and regular visual or physical captures performed until December 1997.

Mitochondrial DNA, allozymes and microsatellite analysis

DNA extractions were performed using a classical phenol–chloroform method. Amplified fragments from the mitochondrial control region were digested using *Rsa*I and electrophoresed on 9% small nondenaturing poly-

acrylamide gel (Mougel 1997). The restriction patterns, revealed by silver staining, distinguish two mitochondrial DNA types: B_1 and $B_{3/4}$. These two mitochondrial types are characteristic of French wild rabbit populations (see Mougel 1997 for B_1 and $B_{3/4}$ specifications). Six allozyme loci (ADA, ALB, CAII, HBA, HBB, PGD) and eight microsatellite loci (sat2, sat3, sat4, sat5, sat7, sat8, sat12, sat13) were analysed, as described, respectively, in Ferrand (1995) and Mougel et al. (1997).

Genetic diversity and bottleneck analysis

Allele frequencies, mean number of alleles per locus (n_{all}) of Table 1, observed heterozygosity (H_O), unbiased expected heterozygosity (H_E) and its standard deviation (Nei 1987) were calculated for allozymes and microsatellites in each of the three samples (1982, 1994, 1996) using GENETIX (Belkhir et al. 1996). Deviations from Hardy–Weinberg expectations, homogeneity tests on allele or genotype distributions and linkage disequilibrium between all loci were tested using GENEPOP (Raymond & Rousset 1995). Linkage disequilibrium is suspected when the test is significant ($P < 0.05$) for the three samples. To reveal possible immigration, foreign genotypes were checked for using GENECLASS (Cornuet et al. 1999). The bayesian algorithm (with the 'as is' procedure)

was used, and the probability that each individual belonged to the population (in the 1996 sample) was calculated with default parameters (10 000 simulated individuals with a reject probability of 0.01).

Standard statistical tests were used to detect bottlenecks (Luikart *et al.* 1998b): (i) a Wilcoxon's signed-rank test for loss of mean heterozygosity; (ii) a Wilcoxon's signed-rank test for loss of mean number of alleles per locus; and (iii) a test for changes in the distribution of allele frequencies (Luikart *et al.* 1998a). This last test consists of comparing distributions established for each sample after grouping the alleles in frequency classes of size 0.1. After a recent bottleneck, a rapid loss of low-frequency alleles (0–0.1) and a relative abundance of intermediate and high-frequency alleles (> 0.1) are expected.

Changes over time in allele frequencies

The change over time in allele frequencies for the 1982–1994 and 1994–1996 periods was computed using GENEPOP (Raymond & Rousset 1995) with an exact homogeneity test. This method had already been used for temporally spaced samples (Waples 1989a; Viard *et al.* 1997). However, the null hypothesis stipulated by this standard statistical test considers two independent binomial samples from the same probability distribution. This standard test is thus not appropriate to determine whether the observed differences in temporally spaced samples are significant because, in this case, allele frequencies are not independent estimates of the same parameter. Therefore, a generalized method was developed to estimate the probability of obtaining a significant test if the only forces acting on allele frequencies are stochastic, i.e. genetic drift and sampling error (Waples 1989a). In the Waples' test, the null hypothesis considers that observed differences in allele frequencies are due entirely to stochastic process in a finite population. Therefore, the conclusion of a significant test result is that observed changes in allele frequencies can be attributed to drift (Waples 1989a).

Analyses were conducted according to sampling plan I (sampled individuals are replaced before reproduction occurs; Waples 1989a) because rabbits were caught and replaced in October–December, whereas their reproduction period ends in September and starts again in January. The temporal method also requires some assumptions about the effective population size (N_e), the number of generations between two samples (t), the sample sizes (S_0, S_t) and the population size (N) at generation 0. Because of the relative inaccuracy of capture–recapture data (and lack of precise demographic data before 1989), we used the extreme values of N (200 and 600) which take into account the likely variation of population size over time (see results on population demography). Generation time (T) is difficult to assess and estimates depend on the popula-

tions studied: $T = 1.17–1.91$ was based on five populations in Australia (Myers *et al.* 1971), $T = 1.8$ on one population in Australia (Daly 1981) and $T = 1.03$ on one population in Spain (Soriguer 1983). The tests were conducted with $T = 1$, because our field studies indicated that the generation time of the Chevreloup population is ≈ 1 year (S. Marchandeau *et al.* unpublished results). Finally, because the effective population size (N_e) is very difficult to estimate from ecological methods (Nunney 1995), the critical value of N_e at and after which the test becomes significant ($P = 0.05$) was calculated.

Failure to reject the null hypothesis (allele frequency changes can be explained by sampling error and drift) is not necessarily a powerful indication that other factors (e.g. selection) are not involved. Selection may have two origins: either the general molecular properties of some studied loci or as a consequence of epizootic. First, at mutation drift equilibrium, selectively neutral loci do not present an excess or deficit in heterozygosity. Comparison between observed and expected homozygosities has been used as a test for detecting selection (Watterson 1978; Paterson 1998). The same test also developed to detect strong recent bottlenecks (Cornuet & Luikart 1996) was applied here to detect, in each sample, the deviations from neutrality at mutation drift equilibrium (specifically for allozyme loci) using BOTTLENECK software (Piry *et al.* 1997). Secondly, during the epizootic (in 1995), nonrandom removal of genotypes could be responsible for changes in allele frequencies. To detect such selective effects, the genotype distributions of two groups of rabbits within the 1994 samples were compared with the help of a homogeneity test performed by the GENEPOP program (Raymond & Rousset 1995). The first group includes rabbits sampled in 1994 which survived the epizootic (observed by spotlighting or caught after 1 January 1996). The second group includes rabbits sampled in 1994 and presumed to have died during 1995 epizootic (because they had not been observed or caught from 1995 to December 1997).

Effective sizes of population

When the changes in allele frequencies can be explained by the stochastic effects alone, the intensity of genetic drift is quantified by estimating the effective population size (N_e) using a genetic method. The temporal method estimates effective population size from the standardized variance in allele frequency change (Waples 1989b). The variance in allele frequencies was calculated for each locus according to Nei & Tajima (1981):

$$F_C = \frac{1}{k}\sum_{i=1}^{k}\frac{(x_i - y_i)^2}{(x_i + y_i)/2 - x_i y_i}$$

where x_i and y_i are the allele frequencies of the ith of k alleles. A mean F_C across all loci was weighted by the number

of alleles at each locus. According to sampling plan I (see above), N_e was estimated using the following formula:

$$N_e = \frac{t}{2(F_C - 1/2S_0 - 1/2S_t + 1/N)}$$

where t is the number of generations between samples, S_0 the sample size at generation 0, S_t the sample size at generation t and N the population size at generation 0. Like in Waples' tests, the extreme values of N (200 or 600) and the generation time of 1 year ($T = 1$) were used. A 95% confidence interval (CI) for N_e was calculated as described by Waples (1989b; eqn 15).

Results

Population demography

With regard to the total population, the estimated census size at the beginning of each breeding season (January) varied from 246 to 543 animals between 1989 and 1994, but decreased to 79 in January 1996 and 39 in January 1997. As suggested by Waples (1989b) when the population size varies over time, harmonic means were calculated: 373 between 1989 and 1994 and 52 between 1995 and 1996. Following the outbreak of RVHD, the size of the subgroup studied on the 5-ha central area decreased from 205 adults to 24 (and from 134 juveniles to 2) at the beginning of 1996 (Fig. 2). The annual mortality rate in 1995 was 88% in adults and 99% in juveniles. About 80% of the warrens (network of burrows) were empty after the epizootic, and the warrens in which rabbits survived were almost evenly spaced (Fig. 1). No migration of untagged rabbits into the studied territory was observed. Furthermore, the probability for each rabbit (taken individually) to have actually been included in the overall 1996 sample was always found to be higher than the probability of rejection ($P > 0.01$, data not shown). In consequence, any hypothetical immigration can be ruled out.

Genetic diversity and genetic structure

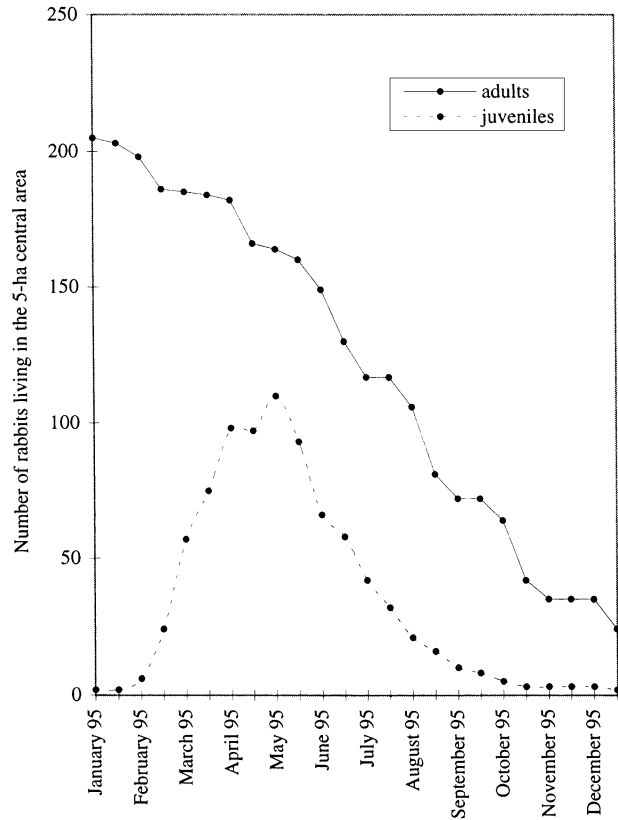Almost no variation was found in mitochondrial DNA

Fig. 2 Temporal changes in the size of the adult and juvenile population living on the 5-ha central area (from Marchandeau *et al.* 1998a).

sequences. Every rabbit, in whatever sample (1982, 1994 and 1996), featured the allele B_1, except for one animal carrying the type $B_{3/4}$ in the 1994 sample. At the nuclear level, no significant differences ($P > 0.05$; Wilcoxon's tests not shown) were observed in the number of alleles per locus or in heterozygosity for the 1982–1994 and 1994–1996 periods for both allozymes and microsatellites (Table 1). The distribution of microsatellite alleles (taken as a whole) into frequency classes is shown for 1982, 1994 and 1996 samples (Fig. 3). No reduction was observed in the

Sample	n	n_{all}	H_O	H_E	p_{HW}
Allozymes (6 loci)					
1982	55	2.33	0.386	0.377 ± 0.196	0.709 NS
1994	45	2.33	0.373	0.357 ± 0.200	0.998 NS
1996	40	2.33	0.365	0.374 ± 0.201	0.311 NS
Microsatellites (8 loci)					
1982	45	4.75	0.613	0.651 ± 0.088	0.098 NS
1994	44	4.75	0.621	0.631 ± 0.096	0.359 NS
1996	50	5.00	0.629	0.622 ± 0.097	0.769 NS

Table 1 Genetic diversity and probability values of rejecting the Hardy–Weinberg expectations (p_{HW}) for allozymes and micro-satellites. Sample sizes (n), mean number of alleles per locus (n_{all}), observed heterozygosity (H_O) and expected heterozygosity (H_E) are presented
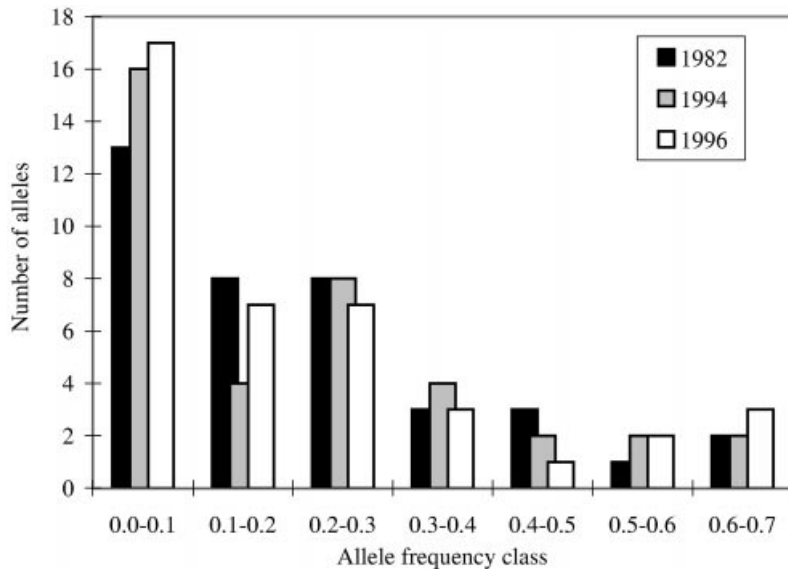
NS, not significant.

Fig. 3 Microsatellite allele frequency distribution for the three samples 1982 (black bars) 1994 (grey bars) and 1996 (white bars). The distribution is established after grouping the alleles of all loci into 0.1 frequency classes.
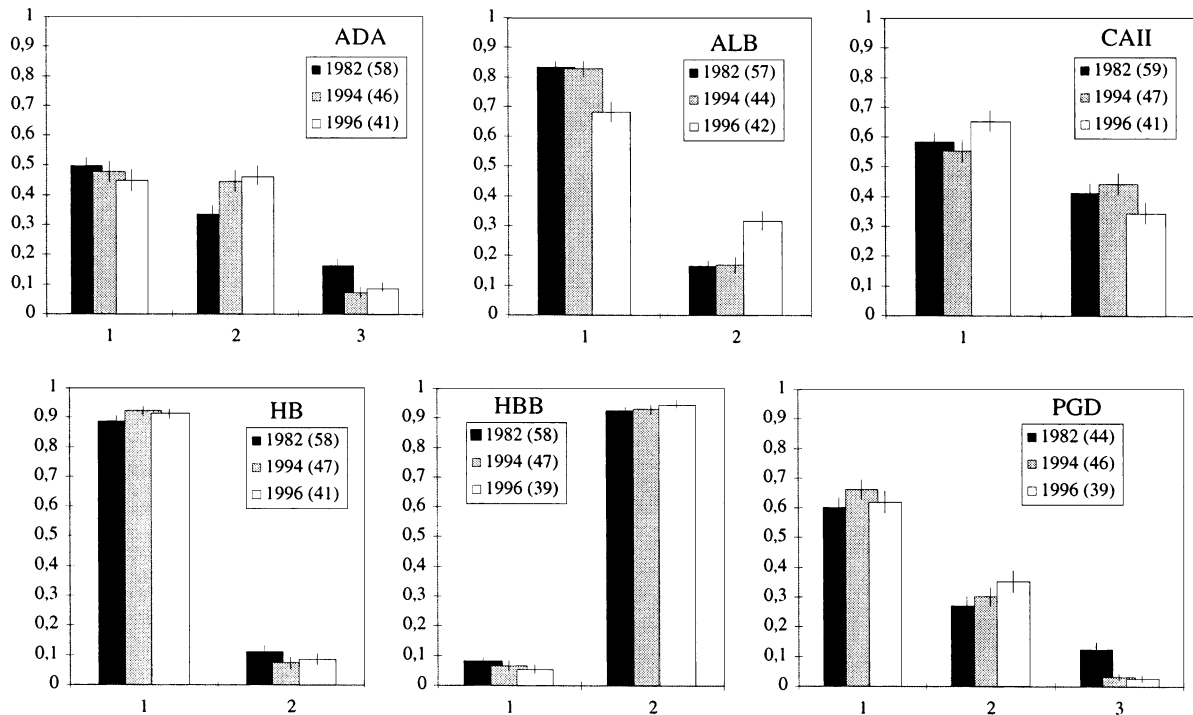

Fig. 4 Distributions of allele frequencies for six allozyme loci in the three samples: 1982 (black) 1994 (grey) 1996 (white). The numbers in parentheses are the sample sizes.

number of alleles in the low allele frequency class between 1994 and 1996 samples. Because of a low genetic diversity and the quasi absence of low frequency alleles, similar analysis was not performed for allozymes. The observed genotype frequencies were in agreement with the Hardy–Weinberg expectations for both allozymes and microsatellites (Table 1) even at the locus level ($P > 0.05$; data not shown). Linkage disequilibrium was found between

sat2 and sat4 microsatellite loci ($P < 0.01$ for the 1982, 1994 and 1996 samples). In order to avoid any possible bias, these two loci were individually removed from further tests.

Temporal variation of allele frequencies

Figures 4 and 5 show the allele frequencies for the respective allozymes and microsatellites. The change in allele
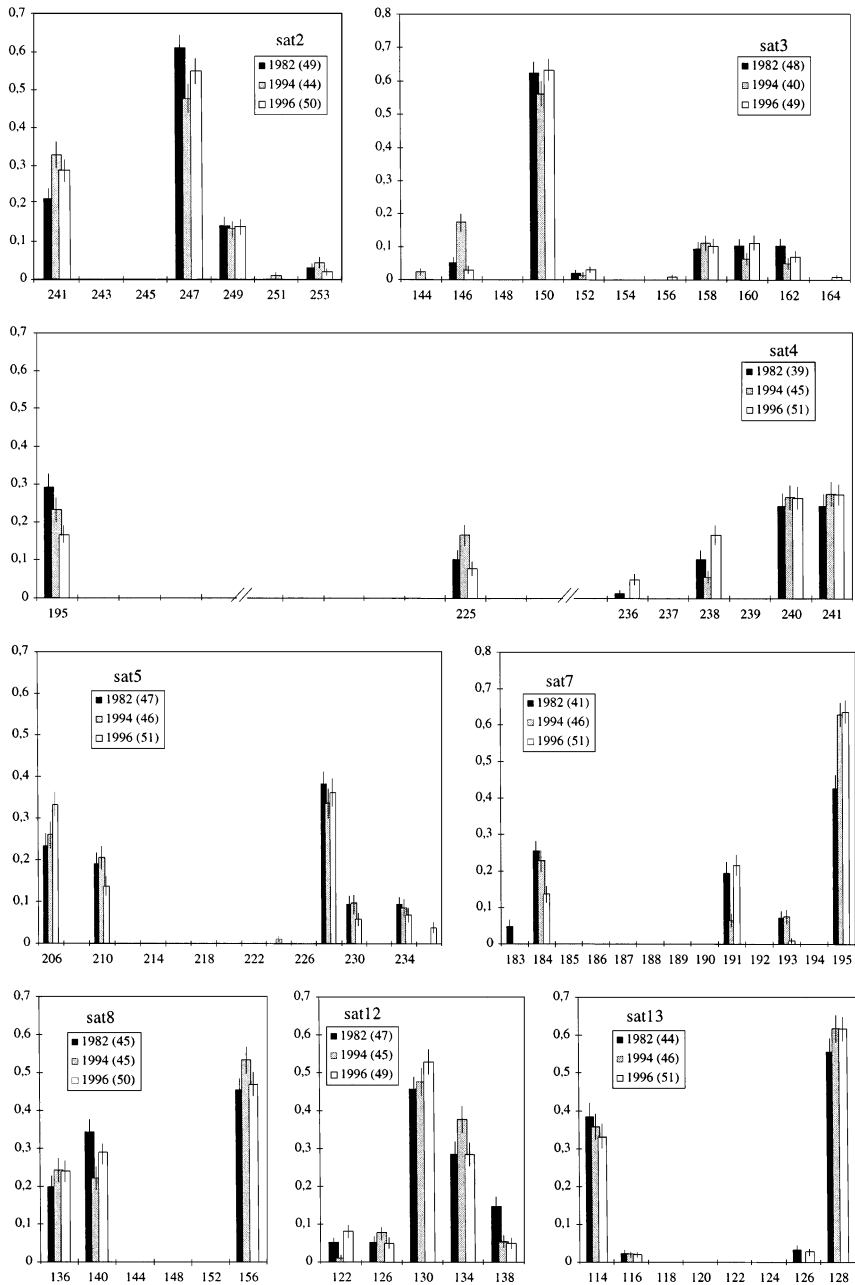
Fig. 5 Distributions of allele frequencies for eight microsatellite loci in the three samples: 1982 (black) 1994 (grey) 1996 (white). The numbers in parentheses are samples sizes.

frequencies over time was examined using an exact homogeneity test (Table 2). For allozymes, no significant differences were observed for the 1982–1994 ($P = 0.529$) and 1994–1996 ($P = 0.369$) periods. In spite of the absence of extensive variations in microsatellite allele frequencies between the 1982, 1994 and 1996 samples (Fig. 5), statistical tests showed a slight significant change ($P = 0.018$) between 1982 and 1994, and a highly significant change ($P = 0.001$) between 1994 and 1996 (Table 2). Much greater changes in allele frequencies were observed in ALB ($P = 0.022$) and sat7 ($P = 0.006$; $P = 0.003$) loci. When the ALB locus

was removed from the analysis, no significant change in allozyme frequencies was found ($P > 0.05$). However, when sat7 was removed, the 1982–1994 changes in microsatellite frequencies were no longer significant ($P = 0.130$) and the level of significance changed for the 1994–1996 period ($P = 0.026$ instead of $P = 0.001$). When the two linked loci (sat2 and sat4) were removed one by one, the changes in microsatellite frequencies were still significant for 1982–1994 ($P < 0.05$) and 1994–1996 ($P < 0.01$).

Waples' temporal test was performed to investigate whether stochastic effects alone (sampling error and genetic

Table 2 Homogeneity tests for temporal changes in allele frequencies for allozymes and microsatellites for the periods 1982–1994 and 1994–1996

	1982–1994	1994–1996
Allozymes		
ADA	0.169	0.874
ALB	1.000	0.022*
CAII	0.671	0.174
HBA	0.647	1.000
HBB	1.000	0.751
PGD	0.070	0.752
6 loci	0.529	0.369
5 loci (— ALB)	0.441	0.891
Microsatellites		
sat2	0.257	0.634
sat3	0.052	0.016*
sat4	0.495	0.016*
sat5	0.968	0.224
sat7	0.006**	0.003**
sat8	0.201	0.560
sat12	0.090	0.134
sat13	0.386	0.505
8 loci	0.018*	0.001**
7 loci (— sat7)	0.130	0.026*
7 loci (— sat2)	0.019*	0.001**
7 loci (— sat4)	0.011*	0.004**
All loci	0.098	0.014*

*$P < 5\%$; **$P < 1\%$.

drift) could explain the heterogeneity in microsatellite loci observed over time. A significant test (at the 0.05 level) implies that pure drift was not sufficient to explain the differences in allele frequencies. The tests became significant for the 1982–1994 period when $N_e > 589$ (with $N = 200$) and $N_e > 884$ (with $N = 600$). The same is true for the 1994–1996 period when $N_e > 81$ (with $N = 200$) and $N_e > 112$ (with $N = 600$). Moreover, the temporal test conducted on allozyme loci is never significant whatever effective size used in the calculations.

Whatever the variation in allele frequencies, loci were tested for their selective effects. First, when taking all allozyme loci and all microsatellite loci together, no deviation from neutral allele frequency distributions was found ($P > 0.05$ for 1982, 1994 and 1996 samples). Second, no significant differences were observed when comparing the genotype distributions of the surviving and presumed-killed rabbits for both allozyme and microsatellite loci ($P > 0.05$).

Effective population sizes

The effective population size (N_e) and its confidence interval were estimated using the temporal method of Waples with $N = 200$ and $N = 600$ (Table 3). Because the changes in allozyme and microsatellite frequencies were attributed to genetic drift, considering both allozymes and microsatellites together led to better estimates of N_e with smaller confidence intervals. When all loci were taken into account, the effective population size was found to be significantly lower for the 1994–1996 period ($N_e = 52–63$) than for the 1982–1994 period ($N_e = 361–451$).

Discussion

Absence of genetic diversity loss

Although the 1995 epizootic led to a high mortality rate (88–99%), no decrease in genetic diversity (allelic diversity and heterozygosity) was detected following the demographic crash, in spite of large sample sizes (≈ 50 animals) and the number of polymorphic loci used. In the absence of confidence intervals for the populations size estimates, one may assume that the demographic decline was not as severe as evaluated. Nevertheless, intense monitoring of the central area (trapping, ferreting and spotlighting) compensated for this absence, leaving us confident about our estimate of the numbers of living rabbits. An immigration following the epizootic could not be responsible either for the absence of genetic diversity loss because no untagged rabbits and no foreign genotypes were detected. Besides, comparison between genotypes of surviving and dead rabbits in 1995 showed no selective effects. An explanation must therefore be found elsewhere.

	Allozymes		Microsatellites		Alloz. + Microsat.	
	N_e	95% CI	N_e	95% CI	N_e	95% CI
1982–1994						
$N = 200$	995	[249; 8]	297	[163; 867]	361	[201; 943]
$N = 600$	2226	[289; 8]	355	[179; 1674]	451	[234; 1981]
1994–1996						
$N = 200$	73	[25; 8]	48	[26; 141]	52	[30; 132]
$N = 600$	96	[27; 8]	57	[29; 266]	63	[34; 237]

Table 3 Estimates of effective population size (N_e) and 95% confidence interval calculated by temporal methods. N is the population size at the beginning of each period. Two values of population size (N) were used for the two periods 1982–1994 and 1994–1996 (see Materials and methods)

In fact, the main explanation for the absence of diversity loss is the remnant population size and the time elapsed after the crash. The high mortality rate (88–99%) is not informative, because the census and effective population sizes were sufficiently high (≈ 50 animals) to retain almost all the genetic diversity. Theoretical predictions about bottlenecks affirm that loss of genetic diversity depends on the minimum population size and growth rate following the decline (Nei *et al*. 1975). Although population bottlenecks of short duration have relatively little effect on hererozygosity, they are predicted to have a larger immediate effect on allelic diversity (Nei *et al*. 1975; Leberg 1992). Nevertheless, Allendorf (1986) has shown that for a constant effective size of 25, a significant loss in allelic diversity is hardly observed before 15 generations. Thus, even if the studied population did not grow after the epizootic, it was considered only two generations after the experienced crash and the effective population size proved to be still relatively high (mean N_e estimates of 43–50). The finding of no detectable reduced variation is consistent with theoritical predications and with other empirical studies. For example, in *Rhinoceros unicornis* (Dinerstein & MacCracken 1990), a demographic bottleneck occurred recently in comparaison with generation time and did not produced a decrease in genetic variability; the number of surviving animals was relatively high (tens of individuals) in spite of strong mortality ($\approx 90\%$). We may conclude that the absolute number of survivors (≈ 50 animals in the present case) is the decisive factor and that the mortality rate is not therefore a good indicator of a suspected genetic bottleneck.

Temporal variation of allele frequencies: differences between allozyme and microsatellite data

Heterogeneity in allele frequencies between the three samples (1982, 1994 and 1996) was observed for microsatellite loci but not for allozymes. Different interpretations could be advanced to explain the absence of change in allozyme frequencies specifically between 1994 and 1996. First, allozyme frequencies changed slighty but their polymorphism was not high enough to detect any significant variation. Second, selective effects (balancing selection for example) could have produced apparent stability in allele frequencies as already described in allozyme loci in some species (Karl & Avise 1992; Bancroft *et al*. 1995). However, stability of allele frequencies could only be explained if the variations due to nonrandom removal of genotypes during epizootic were compensated by a balancing selection in the following generations. With the present data, it was not possible to test such balancing selection, but the random removal of genotypes (for both allozymes and microsatellites) during the epizootic was demonstrated when testing homogeneity between genotype distributions

of the surviving and of the presumed-dead rabbits. For this reason, the balancing selection explanation can be dismissed and the differences in allozyme and microsatellite results are more likely explained by the relatively low polymorphism of allozyme loci and hence their lack of statistical power.

Waples' tests performed on microsatellite loci would become significant if $N_e > 589$ (1982–1994 period) and $N_e > 81$ (1994–1996 period). Because the effective population size (N_e) is probably lower than or equal to the estimated census size (373 for 1982–1994 and 52 for 1994–1996) the tests were not significant. As temporal tests conducted on allozyme and microsatellite loci were not significant, genetic drift and sampling error are a sufficient explanation of the observed temporal changes in allele frequencies, thus confirming the absence of significant selective effects.

Effective population size

The estimated effective population size was found to be near census population size (361–451 vs. 373 for 1982–1994; 52–63 vs. 52 for 1994–1996) and significantly different before and after the epizootic (respectively 52–63 and 361–451). However, we are unable to give a realistic N_e/N ratio because of the uncertainty about the population size before the demographic crash and the large confidence intervals for N_e. Calculation of N_e also depends on generation time: the higher T is ($T > 1$), the more likely the N_e/N ratio is to be found significantly lower than 1 (if $T = 2$, N_e was 26–32 and 180–226 for 1982–94 and 1994–96 periods, respectively).

Because only the expectations under pure drift conditions are of interest in Waples' test, selection, migration and mutation are ignored. Conclusions drawn from this test may be misleading if these assumptions (no selection, no migration, no mutation) are not verified. In the present study, mutation was not supposed to have a strong effect over a relatively short period (12 years), even in the case of microsatellites which exhibit high mutational rates (Goldstein *et al*. 1995; Slatkin 1995). Selective effects were already ruled out for both allozyme and microsatellite loci through comparison between genotypes of surviving and dead rabbits and tests for selective neutrality. Finally, the absence of immigration into the studied population after the demographic crash was demonstrated.

Although discrete generations are assumed in the temporal method, in many cases, the results are also applicable to organisms with overlapping generations (Pollak 1983; Waples 1989a). In age-structured populations, discrete cohorts may, however, differ by their allele frequencies. As temporal shifts in allele frequencies alone are not sufficient to estimate N_e in the case of overlapping generations, Jorde & Ryman (1995) developed a method to estimate effective size by taking into account the age-specific survival and

birth rates of the population. Because of the difficulty in calculating these parameters, it was not possible to use their estimator of effective size. Finally, as the males and females reproduce for 1 or 2 years only (Mykytowycz 1958; Garson 1982), we assumed that the studied population did not differ from a homogeneous breeding group. To conclude, because the assumptions of the temporal method (no selection, no migration, no mutation and no overlapping generations) are most probably fulfilled, the results of temporal changes in allele frequencies and effective population size estimates are acceptable.

Possible effects of social structure

Social behaviour described previously in rabbits may influence the genetic structure and the apportionment of genetic diversity within populations. Rabbit populations are subdivided into small social groups which are known to form hierarchies that determine an individual's breeding success (Mykytowycz & Fullager 1973; Garson 1982; Daly 1981). Genetic differentiation among lineages can be expected when a few polygynous males are breeding with philopatric females (Chesser 1991). Previous studies on rabbit social organization using allozymes did not find any genotypic differentiation between social groups (Daly 1981). Other studies using fingerprinting (Webb *et al.* 1995) and microsatellites (Surridge *et al.* 1999b) conclude that breeding groups constitute genetically differentiated units. These last results are very difficult to assess because of sampling variance when the genetic parameters are calculated for small social groups (< 10 rabbits within each group). Moreover, juvenile dispersal between social groups observed in the studied population (unpublished results) as well as in other studies (Vitale 1989; Künkele & Von-Holst 1996) probably lead to sufficient gene flow among warrens and a homogeneous apportionment of genetic diversity within populations. Here, the studied population approximated a panmictic population, and the social organization probably had no significant effect on the potential loss of genetic diversity and did not lead to a cryptic bottleneck (a higher decrease in the effective population size than in census population size, Luikart *et al.* 1998b).

Conclusion

The 1995 RVHD epizootic resulted in a high mortality rate ($\approx 90\%$) in the French wild rabbit population considered in this study, although the analysis of 14 polymorphic loci (allozymes and microsatellites) evidenced no loss in genetic diversity. The temporal changes in allele frequencies indicated that the population evolved under genetic drift and that its effective size (N_e) was reduced significantly by the RVHD outbreak. Because only a little time went by after the crash (only two generations), the

bottleneck was short and the remnant population size remained high enough (≈ 50 individuals) to keep diversity at the level of before the crash. While this situation should now be confirmed by other investigations in different parts of the distribution range of the species, the present results suggest that, contrary to what is usually thought and in spite of the subsequent high mortality rates, past epizootics (especially myxomatosis) may have had little effect on the genetic diversity of wild rabbit populations in Europe.

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