Population structure and genetic variation of European wild rabbits (*Oryctolagus cuniculus*) in East Anglia

ALISON K. SURRIDGE*, DIANA J. BELL, KAMAL M. IBRAHIM & GODFREY M. HEWITT

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

The European wild rabbit (*Oryctolagus cuniculus*) is an introduced species in Britain, and populations have been profoundly influenced by both man and disease. In stable environmental conditions, distinct social behaviour is observed, and this social structure leads to significant genetic structuring at the intrapopulation level. In this study, European wild rabbits were sampled from 17 sites across the East Anglian region of Britain and genotyped with nine microsatellite loci. Genotypical proportions deviated significantly from Hardy–Weinberg equilibrium, reflecting a degree of population subdivision and non-random mating. Several estimates of measures of population genetic structure revealed that populations are genetically distinct and have small effective population sizes. These distinctive properties are seen to be the combined effects of the social structure and random drift acting on bottlenecked populations after myxomatosis. It is concluded that the genetic structure seen in rabbit populations today is unlikely to reflect historical structuring present before myxomatosis, but that it results from recent events.

Keywords: effective population size, European wild rabbit, gene flow, microsatellites, *Oryctolagus cuniculus*, population bottleneck.

Introduction

The European wild rabbit (*Oryctolagus cuniculus*) originated in southern Spain and north Africa and now has a widespread global distribution, primarily because of the influence of man. It was introduced into Britain in the eleventh century by the Normans, who kept captive populations of rabbits bred as a food and fur resource. Wild populations were rare owing to lack of a suitable habitat and an abundance of predators. In the eighteenth century, the growing of winter crops combined with an increased interest in game resulted in food resources becoming available and in the control of predators. This meant that populations could survive and increase in the wild (Sheail, 1971). Within 200 years, populations had expanded over most of the UK. In 1952, the myxoma virus was introduced to France in an attempt to control rapidly increasing populations and, by 1953, the virus had spread to Britain where it caused an initial mortality of up to 99.9%. Although rabbits have developed some immunity to the disease, myxo-

matosis is still thought to have a considerable impact on population sizes.

Within the East Anglian region of Britain, the history of the establishment and distribution of rabbit warrens is well documented. Before the sixteenth century, rabbit warrening was largely restricted to areas of light sandy soil (Bailey, 1991). Many areas of forest and heavy soil were inaccessible to rabbits until as late as the eighteenth or nineteenth centuries when land use began to change, and populations of wild rabbits became established.

The European wild rabbit is a burrowing animal that has evolved coloniality, the benefits of which include increased protection from predators, shelter and availability of nesting sites provided by the group warren, along with desirable changes in the quality and quantity of food produced by group foraging effects (Bell, 1983). Within this system, small, stable breeding groups are formed, and linear dominance hierarchies are observed in both males and females, with dominant individuals generally exhibiting higher reproductive success (Bell, 1983). Natal dispersal is sex biased; males disperse and females tend to remain within their breeding group (Webb *et al.*, 1995). This social structuring within a population of European wild rabbits is seen to result in

*Correspondence. E-mail: a.surridge@uea.ac.uk

higher relatedness among females within a social group than among males (Surridge *et al.*, 1999). An overall reduction in gene flow is observed, leading to genetic structuring of the population, with breeding groups constituting genetically isolated units.

The fine-scale genetic structuring resulting from social behaviour may be expected to have a significant impact on the larger scale population structure of the European wild rabbit. This study investigates the genetic structure of rabbit populations in the East Anglian region of Britain using nine polymorphic microsatellite loci, with the aims of examining: (i) the wider scale influences of social behaviour; (ii) the historical effects of founder events as populations expanded across the East Anglian region; and (iii) the influence of the myxomatosis bottleneck on population genetic structure. Populations from areas where rabbits were established initially were sampled together with areas where rabbit populations would have become established around 700 years later. The influence of population bottlenecks and founder effects was assessed by estimating genetic diversity within these different populations.

Materials and methods

Sampling

Seventeen sites across East Anglia were sampled; between 20 and 55 individual rabbits were collected from each site. The approximate defended home territory of a rabbit is 0.25 ha; samples for this study were collected from areas of up to several hundred hectares. The distribution of sample sites across East Anglia is given in Fig. 1. DNA was extracted from small pieces of ear tissue taken from rabbits shot as part of routine control procedures. Ear tissue was preserved in a high-salt tissue preservation buffer.

Genetic analysis

DNA extraction was performed using standard techniques (overnight cell lysis using proteinase K, SDS and EDTA; protein purification using chloroform followed by isopropanol precipitation) and polymerase chain reaction (PCR) amplification and genotyping of microsatellite loci performed as described previously for the nine loci: *sol03*, *sol08*, *sol30*, *sol33*, *sol44*, *sat5*, *sat7*, *sat8* and *sat12* (Surridge *et al.*, 1997).

Statistical analysis

First, we estimated the genetic diversity within the populations sampled using the Shannon–Weaver diver-
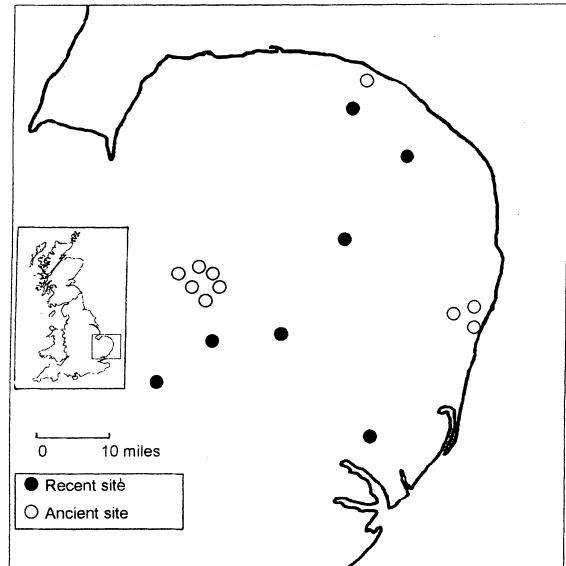

Fig. 1 Distribution of sample sites of European wild rabbit populations in East Anglia. Recent and ancient sites are marked by closed and open circles, respectively.

sity index (Shannon & Weaver, 1964). This index integrates two aspects of diversity, variant richness as well as the frequency of each variant, i.e. the number of alleles and their frequencies. For an infinite population, diversity is estimated by $H' = -\sum p_i \ln p_i$, where p_i is the frequency of variant i, in this case allele i. The bias in H' resulting from finite sample size is small and can be ignored in most cases (Peet, 1974). H' is maximized when each individual sampled carries unique alleles; thus, maximum H' is given by $\ln n$, where n is the total number of alleles sampled. Relative diversity was calculated as the ratio of H' to maximum H'. These diversity indices should reveal the effects of past bottlenecks and/or the founding of populations from a small number.

Secondly, we tested the genotypes at each locus for each population for deviation from the expected Hardy–Weinberg ratios using the software POPGENE (Yeh *et al.*, 1996), which performs both chi-squared and likelihood ratio tests of statistical significance. Rabbits show a high degree of social structure and, therefore, it is unlikely that the genotypical proportions in the sampled populations will conform to Hardy–Weinberg expectations. However, assuming that any heterozygote deficit found is caused entirely by the effects of null alleles, it is possible to calculate the frequency of these null alleles from the expected (H_e) and observed (H_o) heterozygosity values. This can be given by:

$$r = H_e - H_o / (H_e + H_o) \quad \text{(Chakraborty *et al.*, 1992)} \quad (1)$$

and $r = H_e - H_o/(1 + H_e)$ (Brookfield, 1996),　　(2)

where r is the frequency of null alleles.

　　Thirdly, Wright's (1951) F_{ST}, F_{IT} and F_{IS} (based on an infinite allele model of mutation) were estimated in the form of θ, F and f, the sample-based, respective estimators of these parameters proposed by Weir & Cockerham (1984). These were computed using FSTAT (Goudet, 1995). Because there is no general consensus over which model of mutation is best applied to microsatellite data (Di Rienzo *et al.*, 1994), we also obtained estimates of Slatkin's (1995) R_{ST} (based on a stepwise mutation model) in the form of ϕ_{ST} values calculated using AMOVA (Michalakis & Excoffier, 1996). Pairwise genetic distances (in the form of θ) were plotted against pairwise geographical distances in kilometres. A Mantel test (Mantel, 1967) was used to test for a significant relationship.

　　Also estimated were Nm or gene flow in the form of number of migrants exchanged per generation, and N_e, the effective population size. The relationship $Nm = (1/4F_{ST}) - 0.25$ (Wright, 1951) was used to estimate the number of migrants per generation from θ, the estimator of F_{ST} (where equilibrium conditions in terms of the effective size of a population over generations and the balance between drift and migration are assumed). Slatkin's (1985) method of estimating Nm, based on the distribution of rare or private alleles, was calculated (with refinements) in GENEPOP (Raymond & Rousset, 1995).

　　Similarly, where steady state, neutrality and an infinite allele model are assumed, Ewens (1972) has derived the expectation of the mean number of different alleles per locus in a sample as a function of the rate of mutation μ, sample size n and the effective population size N_e. Thus, for a given sample size and observed number of alleles, the value of $4N_e\mu$ was obtained, and N_e was calculated assuming a mutation rate of 10^{-3}, which lies between reported rates for microsatellites in rodents and humans (Dallas, 1992; Weber & Wong, 1993). The formulae for N_e derived by Crow & Kimura (1970) and Ohta & Kimura (1973) (where N_e is a function of μ and the observed heterozygosities for the infinite and single-step stepwise mutation models respectively) were used to obtain N_e values for comparative purposes.

Results

Microsatellite loci

All the microsatellite loci showed polymorphism, having between eight and 17 alleles and heterozygosities ranging from 0.24 to 0.72 (Table 1). The mean number of alleles per locus ranged from 3.45 to 7.78 in the 17

Table 1 Number of alleles, observed and expected heterozygosities for the nine microsatellite loci of European wild rabbits

Locus	No. of alleles	H_o	H_e	r_1	r_2
sol03	17	0.627	0.891	0.174	0.140
sol08	11	0.573	0.818	0.176	0.135
sol30	15	0.619	0.821	0.140	0.111
sol33	16	0.581	0.855	0.191	0.148
sol44	15	0.241	0.729	0.503	0.282
sat5	17	0.523	0.694	0.141	0.101
sat7	16	0.471	0.827	0.274	0.195
sat8	8	0.326	0.404	0.107	0.056
sat12	9	0.718	0.748	0.020	0.017

Also given are frequencies of null alleles (r) expected under departure from Hardy–Weinberg equilibrium using eqns (1) and (2) in the text.

populations. Loci showing the greatest number of alleles tended to be interrupted repeats, for example *sol03* (17 alleles), *sat5* (17 alleles), *sol33* (16 alleles) and *sol30* (15 alleles). However, there appeared to be no clear correlation between number of repeats and number of alleles in our sample of microsatellite loci. Population-specific allele frequencies for all loci are given in the Appendix.

Departures from Hardy–Weinberg equilibrium

The social structure of the European wild rabbit prevents random mating. Reflecting this, all loci were seen to deviate significantly from Hardy–Weinberg equilibrium using both chi-squared and likelihood ratio tests ($P < 0.001$). Observed and expected heterozygosities are given in Table 1, together with the numbers of alleles observed per locus. All loci except *sat12* showed a significant heterozygote deficit. Possible reasons for this deficit, apart from population subdivision and assortative mating, include selection against heterozygotes and null alleles. Estimates of r for each locus are given in Table 1 (r_1 from eqn 1, r_2 from eqn 2). These frequencies are high for some loci, e.g. *sol44* and *sat7*. The frequency of blank genotypes in the data set (possible null–null homozygote genotypes) ranged from 0.022 (for *sat12*) to 0.052 (for *sat7*).

Population differentiation and gene flow

The estimators of population differentiation based on the infinite alleles and stepwise mutation models showed a high degree of population differentiation ($\theta = 0.150$, $P < 0.001$; $\phi_{ST} = 0.198$, $P < 0.001$). Single-locus estimates of F, f, θ and ϕ_{ST} are given in Table 2. All loci show values of θ and ϕ_{ST} significantly greater than zero ($P < 0.005$). Estimates of gene flow, Nm, calculated from θ and ϕ_{ST} are 1.42 and 1.01 respectively. Using the

Table 2 Single-locus estimates of F, f, θ and ϕ_{ST} for the nine microsatellite loci in European wild rabbits

Locus	F	f	θ	ϕ_{ST}
sol03	0.303***	0.199***	0.130***	0.198***
sol08	0.304***	0.212***	0.116***	0.247***
sol30	0.252***	0.157***	0.113***	0.169***
sol33	0.327***	0.225***	0.132***	0.396***
sol44	0.684***	0.507***	0.359***	0.314***
sat5	0.258***	0.085***	0.189***	0.061***
sat7	0.436***	0.348***	0.135***	0.424***
sat8	0.199***	0.126***	0.083***	0.076**
sat12	0.047*	−0.036	0.080***	0.171***
All loci	0.319***	0.199***	0.150***	0.198***

*$P < 0.05$, **$P < 0.005$, ***$P < 0.001$.

private alleles method, a higher value of 2.82 individuals per generation was obtained.

Plots of pairwise θ against geographical distance (Fig. 2) showed no apparent correlation between genetic and geographical distance (Mantel test, $P > 0.05$).

Effective population size

For a value of $\mu = 10^{-3}$, we obtain estimates of effective population size of 541 using Ewens' formula, where the number of alleles per locus was averaged over loci and over populations. The methods based on heterozygosity values gave estimates of 253 and 380 for the infinite alleles and stepwise mutation models, respectively, where heterozygosities were averaged over loci and over populations.
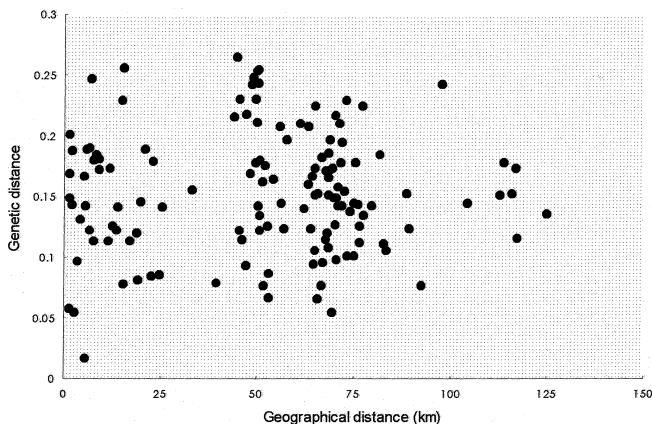

Fig. 2 Plot of pairwise geographical distance (km) against pairwise genetic distance (θ) for the 17 populations of European wild rabbit.

Diversity within populations

The Shannon–Weaver diversity index obtained for populations ranged from 0.92 to 1.79. Relative diversity ranged from 0.082 to 0.136. Of the populations sampled, 10 could be considered 'ancient' populations, i.e. rabbit populations were known from historical records to be present in those areas before the sixteenth century (M. Bailey, personal communication), and the remaining seven were sampled from areas probably colonized by rabbits from the eighteenth century onwards, termed 'recent' populations. As rabbits spread out from existing populations into previously unoccupied habitats in the eighteenth or nineteenth centuries, we might expect to see a loss of genetic diversity resulting from a small number of initial founders. Mean diversity for recent populations = 0.112 (range 0.082–0.125) and, for ancient populations, mean diversity = 0.114 (range 0.096–0.136). There is no significant difference between these two sets of values. These reveal that there is no relationship between the site of the population (ancient site or recent site) and the diversity found within the population. Hence, the data show no evidence for founder effects in populations occupying areas radiating out from sites supporting ancient populations.

Discussion

The European wild rabbit exhibits a high degree of non-random mating and social structuring, and a consequence of this is a high degree of genetic structuring within a population (Surridge *et al.*, 1999). When dealing with sampled populations, therefore, it must be considered that Hardy–Weinberg assumptions do not apply, and that samples are being taken across genetically isolated breeding subunits. This is reflected in the data presented here, a marked effect being a large deviation from expected Hardy–Weinberg genotype frequencies in the form of a heterozygote deficit. Thus, the combined effect of sampling across many breeding subunits, within which individuals are known to mate assortatively, is the major cause of the observed heterozygote deficit (Surridge *et al.*, 1999). Null alleles could also be contributing to the deficit observed, but the frequency at which they occur remains uncertain.

A high degree of population differentiation is observed between the 17 populations, indicating that the populations are substantially isolated. Because of the low levels of gene flow, there appears to be no significant trend of isolation by distance. A wide spread of pairwise genetic distances is observed, which cannot be correlated with geographical distance.

These data are in good agreement with the results of previous studies on genetic structure of geographical populations of European wild rabbits. Richardson *et al.* (1980) found that there was significant variation in local populations of rabbits in New South Wales using allozyme data, but this variation showed no clear pattern and was attributed to drift because of small effective population sizes. In contrast, rabbit populations in an arid region of Queensland show no genetic differentiation and high levels of gene flow over 1600 km². Moving into a semiarid ecosystem, populations become more structured (Fuller *et al.*, 1997). This difference can be explained by differences in ecology and demography. It has been shown that the genetic structure of eastern cottontail populations (*Sylvilagus floridanus*) is not solely a result of social structure (Scribner & Chesser, 1993). In fact, it is the influence of environmental parameters that determine the social behaviour, which, in turn, influences the genetic structure. Gene flow between populations may be increased in three ways: population expansion in favourable conditions; successful dispersal into recently occupied areas after a population crash (Daly 1979); and mass emigration when resource shortage develops. So, we may suggest that, in favourable, more stable conditions such as those experienced in Britain, strict, stable social organization develops, leading to reduced gene flow and small effective population sizes. If effective population size is small, this is expected to lead to changes in the genetic structure of a population because of random drift and, hence, distinct population differentiation.

Estimates of effective population size corresponded well with others obtained previously for the European wild rabbit. From ecological studies, a figure of 120 has been obtained (Daly, 1979) and, using an isolation by distance calculation from distance moved between birth and breeding, Richardson (1981) estimated an effective population size of 170. Over non-arid regions of Australia, effective population size has been calculated at between 220 and 340 using coalescence theory (Zenger, 1996), showing close agreement with our estimates of 253–380 based on heterozygosity under an infinite alleles model and a stepwise mutation model. However, caution must be applied when interpreting estimates of effective population sizes obtained using models that have certain assumptions, such as fixed population size and random mating, which are unrealistic in a species displaying pronounced social behaviour such as the European wild rabbit.

Our data show no evidence for founder effects reducing genetic variation within populations as rabbits expanded into unoccupied habitats. A possible reason for this is the effects of the population bottleneck experienced as rabbit populations were exposed to myxomatosis later on in their history. The resulting survivors in a population would have undergone loss or fixation of different alleles because of drift, leading to genetic differentiation of geographically isolated populations and the obscuring of much genetic structure induced by past events.

It is often assumed that, when a population goes through a severe bottleneck, random genetic drift will induce a massive loss of genetic variability. Despite the extremely large mortality induced by myxomatosis, much variation is still observed within rabbit populations, with average heterozygosity being 0.520. The reduction in heterozygosity induced by a population bottleneck depends not only on bottleneck size but also on the rate of population growth after the bottleneck, with rapid growth limiting the severe effects of drift to a few generations. However, the loss of alleles is largely dependent on bottleneck size only (Nei *et al.*, 1975), because the bottleneck tends to eliminate many low-frequency alleles from the population. It is proposed that the rabbit's short generation time, together with high fecundity leading to a rapid population growth rate, could account for the degree of heterozygosity observed in present populations, despite the severity of the bottleneck. On the other hand, despite the degree of variation still observed in European wild rabbit populations, there can be no doubt that a disease such as myxomatosis resulting in the death of 99.9% of a population must have a significant effect on the genetic structure of that population.

To conclude, the present-day distribution and abundance of the European wild rabbit has been greatly modified by both man and disease. The genetic structure of populations reflects the social structure of the rabbit and is influenced by genetic drift. Hence, any genetic structuring present in European wild rabbit populations before the myxomatosis outbreak, for example influences of man, such as introductions or translocations, or those induced by natural colonization of populations into unoccupied habitats, is not evident in present populations.

Acknowledgements

References

BAILEY, M. 1991. The rabbit and the Mediaeval East Anglian economy. *Agric. Hist. Rev.*, **36**, 1–20.

BELL, D. J. 1983. Mate choice in the European rabbit. In: Bateson, P. P. (ed.) *Mate Choice*, pp. 211–223. Cambridge University Press, Cambridge.

BROOKFIELD, J. F. Y. 1996. A. simple new method for estimating null allele frequency from heterozygote deficiency. *Mol. Ecol.*, **5**, 453–455.

CHAKRABORTY, R., DE ANDRADE, M., DAIGER, S. P. AND BUDOWLE, B. 1992. Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Ann. Hum. Genet.*, **56**, 45–57.

CROW, J. F. AND KIMURA, M. 1970. *An Introduction to Population Genetics Theory*. Harper and Row, New York.

DALLAS, J. F. 1992. Estimation of microsatellite mutation rates in recombinant strains of mouse. *Mamm. Genome*, **3**, 452–456.

DALY, J. 1979. *The Ecological Genetics of the European Wild Rabbit (*Oryctolagus cuniculus *(L.)) in Australia*. Ph.D. Thesis, Australian National University.

DI RIENZO, A., PETERSON, A. C., GARZA, J. C., VALDES, A. M., SLATKIN, M. AND FREIMER, N. B. 1994. Mutational processes of simple-sequence repeat loci in human populations. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 3166–3170.

EWENS, W. J. 1972. The sampling theory of selectively neutral alleles. *Theor. Pop. Biol.*, **3**, 87–112.

FULLER, S. J., WILSON, J. C. AND MATHER, P. B. 1997. Patterns of differentiation among wild rabbit populations *Oryctolagus cuniculus* L. in arid and semiarid ecosystems of north-eastern Australia. *Mol. Ecol.*, **6**, 145–153.

GOUDET, J. 1995. FSTAT, version 1.2: a computer program to calculate *F*-statistics. *J. Hered.*, **86**, 485–486.

MANTEL, N. 1967. The detection of disease clustering and a generalised regression approach. *Cancer Res.*, **27**, 209–220.

MICHALAKIS, Y. AND EXCOFFIER, L. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics*, **142**, 1061–1064.

NEI, M., MARUYAMA, T. AND CHAKRABORTY, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

OHTA, T. AND KIMURA, M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genet. Res.*, **82**, 201–204.

PEET, R. K. 1974. The measurement of species diversity. *Ann. Rev. Ecol. Syst.*, **5**, 285–307.

RAYMOND, M. AND ROUSSET, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.*, **86**, 248–249.

RICHARDSON, B. J. 1981. The genetic structure of rabbit populations. In: Myers, K. and MacInnes, C. D. (eds) *Proceedings of the World Lagomorph Conference*, pp. 37–52. University of Guelph, Guelph.

RICHARDSON, B. J., ROGERS, P. M. AND HEWITT, G. M. 1980. Ecological genetics of the wild rabbit in Australia. II. Protein variation in British, French and Australian rabbits and the geographical distribution of the variation in Australia. *Aust. J. Biol. Sci.*, **33**, 371–383.

SCRIBNER, K. T. AND CHESSER, R. K. 1993. Environmental and demographic correlates of spatial and seasonal genetic structure in the eastern cottontail (*Sylvilagus floridanus*). *J. Mammal.*, **74**, 1026–1044.

SHANNON, C. E. AND WEAVER, W. 1964. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.

SHEAIL, J. 1971. *Rabbits and their History*. David and Charles, Newton Abbott.

SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution*, **39**, 53–65.

SLATKIN, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

SURRIDGE, A. K., BELL, D. J., RICO, C. AND HEWITT, G. M. 1997. Polymorphic microsatellite loci in the European wild rabbit (*Oryctolagus cuniculus*) are also amplified in other lagomorph species. *Anim. Genet.*, **28**, 302–305.

SURRIDGE, A. K., IBRAHIM, K. M., BELL, D. J., WEBB, N. J., RICO, C. and HEWITT, G. M. 1999. Fine-scale genetic structuring in a natural population of European wild rabbits (*Oryctolagus cuniculus*). *Mol. Ecol.*, **8**, 299–307.

WEBB, N. J., IBRAHIM, K., BELL, D. J. AND HEWITT, G. M. 1995. Natal dispersal and genetic structure in a population of the European wild rabbit (*Oryctolagus cuniculus*). *Mol. Ecol.*, **4**, 239–247.

WEBER, J. L. AND WONG, C. 1993. Mutation of human short tandem repeats. *Hum. Mol. Genet.*, **2**, 1123–1128.

WEIR, B. S. AND COCKERHAM, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

WRIGHT, S. 1951. The genetical structure of populations. *Ann. Eugen.*, **15**, 323–354.

YEH, F. C., RONGCAI, Y., MAO, J., ZHIHONG, Y. AND BOYLE, T. J. B. 1996. POPGENE, *the Microsoft Windows-based user-friendly software for population genetics analysis of co-dominant and dominant markers and quantitative traits*. Department of Natural Resources, University of Alberta, Alberta, Canada.

ZENGER, K. 1996. *The Genetic Variation and Evolution of the Mitochondrial DNA Non-Coding Region in Australian Wild Rabbit Populations (*Oryctolagus cuniculus *(L.))*. M.Sc. Thesis, University of Western Sydney.

Appendix

Allele frequencies at nine microsatellite loci for the 17 European wild rabbit populations

Locus	Allele (bp)	Population																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
sol30	137	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	143	0.000	0.015	0.222	0.051	0.052	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.045	0.381	0.238	0.000	0.112
	145	0.474	0.324	0.194	0.296	0.034	0.167	0.176	0.081	0.000	0.017	0.232	0.184	0.227	0.190	0.262	0.090	0.375
	147	0.205	0.294	0.037	0.082	0.397	0.222	0.284	0.468	0.304	0.200	0.268	0.342	0.091	0.000	0.000	0.205	0.087
	149	0.000	0.000	0.000	0.031	0.017	0.000	0.000	0.000	0.000	0.000	0.036	0.026	0.023	0.000	0.000	0.385	0.013
	151	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
	153	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.013
	155	0.000	0.000	0.028	0.082	0.000	0.000	0.014	0.000	0.000	0.000	0.018	0.000	0.000	0.024	0.071	0.000	0.013
	157	0.051	0.044	0.157	0.031	0.034	0.000	0.068	0.016	0.000	0.083	0.161	0.158	0.068	0.000	0.095	0.013	0.050
	159	0.000	0.029	0.000	0.092	0.103	0.000	0.054	0.048	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.064	0.213
	161	0.244	0.162	0.333	0.276	0.069	0.222	0.392	0.387	0.500	0.650	0.268	0.289	0.545	0.405	0.333	0.000	0.050
	163	0.000	0.059	0.000	0.041	0.293	0.389	0.000	0.000	0.022	0.017	0.000	0.000	0.000	0.000	0.000	0.192	0.075
	165	0.026	0.074	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	169	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	171	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
sol03	218	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.161	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	221	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.468	0.348	0.000	0.196	0.025	0.000	0.000	0.000	0.000	0.000
	223	0.053	0.063	0.000	0.019	0.000	0.000	0.013	0.000	0.000	0.067	0.321	0.175	0.024	0.000	0.000	0.000	0.013
	225	0.039	0.016	0.067	0.142	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.363	0.200
	227	0.289	0.125	0.240	0.321	0.328	0.278	0.250	0.000	0.022	0.017	0.000	0.075	0.214	0.250	0.237	0.438	0.237
	229	0.184	0.266	0.096	0.047	0.086	0.111	0.125	0.016	0.022	0.133	0.000	0.275	0.143	0.250	0.184	0.000	0.000
	231	0.039	0.000	0.000	0.009	0.017	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	233	0.000	0.000	0.000	0.000	0.017	0.000	0.013	0.242	0.413	0.017	0.161	0.100	0.000	0.000	0.000	0.000	0.000
	235	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.117	0.143	0.075	0.000	0.000	0.000	0.000	0.000
	237	0.013	0.031	0.010	0.094	0.017	0.000	0.050	0.032	0.043	0.000	0.179	0.000	0.024	0.000	0.000	0.025	0.038
	239	0.053	0.078	0.029	0.000	0.052	0.000	0.112	0.000	0.000	0.067	0.000	0.050	0.024	0.050	0.079	0.025	0.000
	241	0.026	0.094	0.154	0.292	0.086	0.000	0.125	0.016	0.043	0.000	0.000	0.025	0.048	0.000	0.079	0.150	0.213
	243	0.211	0.156	0.106	0.047	0.379	0.611	0.225	0.000	0.000	0.517	0.000	0.150	0.286	0.000	0.053	0.000	0.188
	245	0.013	0.047	0.288	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.190	0.425	0.316	0.000	0.112
	247	0.079	0.125	0.000	0.009	0.017	0.000	0.000	0.000	0.000	0.050	0.000	0.050	0.024	0.025	0.053	0.000	0.000
	257	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sol44	178	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	192	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	194	0.000	0.000	0.000	0.000	0.000	0.000	0.141	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	196	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	198	0.013	0.000	0.000	0.000	0.000	0.000	0.218	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000
	200	0.013	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.000	0.000	0.000	0.026	0.000	0.000
	202	0.090	0.000	0.009	0.010	0.000	0.000	0.192	0.226	0.083	0.150	0.204	0.175	0.000	0.045	0.105	0.000	0.053
	204	0.051	0.000	0.660	0.000	0.000	0.000	0.051	0.081	0.063	0.033	0.185	0.750	0.432	0.068	0.237	0.013	0.816
	206	0.231	0.088	0.217	0.694	0.034	0.222	0.359	0.677	0.792	0.550	0.426	0.000	0.545	0.523	0.368	0.782	0.079
	208	0.590	0.912	0.104	0.296	0.966	0.722	0.026	0.016	0.042	0.267	0.093	0.075	0.023	0.000	0.053	0.179	0.039
	210	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
	218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.364	0.211	0.000	0.000
	222	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000

Appendix (*contd.*)

Locus	Allele (bp)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
sol08	104	0.000	0.103	0.010	0.000	0.036	0.000	0.066	0.113	0.023	0.017	0.308	0.026	0.190	0.000	0.025	0.015	0.000
	106	0.026	0.074	0.082	0.019	0.250	0.389	0.118	0.065	0.273	0.000	0.308	0.553	0.357	0.000	0.250	0.088	0.237
	108	0.679	0.426	0.337	0.173	0.000	0.056	0.487	0.452	0.341	0.250	0.269	0.132	0.119	0.425	0.425	0.265	0.434
	110	0.000	0.015	0.010	0.019	0.000	0.000	0.000	0.081	0.000	0.000	0.077	0.158	0.167	0.000	0.025	0.000	0.026
	112	0.013	0.000	0.031	0.048	0.000	0.000	0.158	0.177	0.000	0.033	0.019	0.000	0.095	0.275	0.025	0.029	0.039
	114	0.064	0.250	0.051	0.087	0.357	0.333	0.026	0.016	0.000	0.000	0.019	0.105	0.071	0.000	0.000	0.000	0.026
	116	0.192	0.029	0.276	0.269	0.357	0.222	0.132	0.065	0.364	0.300	0.000	0.000	0.000	0.075	0.025	0.191	0.092
	118	0.000	0.000	0.061	0.048	0.000	0.000	0.013	0.032	0.000	0.067	0.000	0.026	0.000	0.000	0.000	0.059	0.013
	120	0.013	0.103	0.061	0.308	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.200	0.225	0.353	0.026
	122	0.013	0.000	0.082	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.105
	124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000
sol33	189	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	191	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	193	0.079	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	195	0.329	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	197	0.184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
	199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.025	0.000	0.000
	201	0.092	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.025	0.000	0.000
	203	0.039	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.050	0.075	0.000	0.000
	205	0.000	0.015	0.356	0.167	0.000	0.000	0.051	0.000	0.042	0.000	0.143	0.000	0.136	0.250	0.250	0.128	0.013
	207	0.026	0.176	0.087	0.315	0.172	0.000	0.090	0.233	0.333	0.367	0.214	0.289	0.091	0.000	0.050	0.205	0.276
	209	0.184	0.250	0.327	0.167	0.603	0.667	0.000	0.017	0.104	0.167	0.018	0.079	0.068	0.425	0.125	0.038	0.039
	211	0.039	0.088	0.067	0.278	0.052	0.111	0.423	0.283	0.271	0.217	0.446	0.316	0.205	0.000	0.050	0.436	0.276
	213	0.000	0.074	0.000	0.000	0.086	0.111	0.000	0.117	0.000	0.017	0.125	0.053	0.068	0.025	0.000	0.038	0.026
	215	0.000	0.000	0.096	0.009	0.000	0.111	0.013	0.017	0.000	0.067	0.018	0.026	0.136	0.200	0.375	0.038	0.013
	217	0.000	0.059	0.029	0.037	0.086	0.000	0.423	0.317	0.250	0.167	0.036	0.237	0.182	0.000	0.000	0.103	0.355
	219	0.000	0.338	0.000	0.028	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.013	0.000
sat12	114	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	118	0.064	0.000	0.028	0.058	0.052	0.000	0.013	0.000	0.043	0.017	0.018	0.000	0.000	0.000	0.000	0.013	0.051
	122	0.103	0.147	0.085	0.144	0.069	0.222	0.329	0.086	0.065	0.310	0.321	0.225	0.273	0.190	0.214	0.487	0.603
	126	0.628	0.441	0.236	0.385	0.362	0.444	0.434	0.276	0.217	0.500	0.518	0.425	0.477	0.476	0.429	0.269	0.282
	130	0.090	0.324	0.321	0.067	0.103	0.056	0.184	0.379	0.283	0.138	0.071	0.125	0.091	0.143	0.143	0.128	0.051
	134	0.103	0.074	0.113	0.308	0.379	0.278	0.013	0.207	0.130	0.017	0.071	0.225	0.159	0.190	0.167	0.077	0.000
	138	0.013	0.015	0.038	0.029	0.034	0.000	0.026	0.052	0.261	0.017	0.000	0.000	0.000	0.000	0.024	0.026	0.013
	142	0.000	0.000	0.160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000
	146	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sat5	195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039
	199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
	201	0.000	0.000	0.010	0.087	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.100	0.000	0.000	0.024	0.125	0.066
	203	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.017	0.000	0.150	0.296	0.525	0.023	0.000	0.000	0.125	0.000
	205	0.750	0.632	0.462	0.365	0.569	0.889	0.878	0.567	0.500	0.267	0.278	0.025	0.818	0.841	0.738	0.050	0.711
	207	0.000	0.000	0.010	0.067	0.000	0.000	0.014	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.300	0.000
	209	0.053	0.088	0.365	0.212	0.362	0.056	0.014	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000
	211	0.000	0.000	0.038	0.125	0.000	0.000	0.027	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	221	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
	223	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.241	0.200	0.000	0.000	0.095	0.000	0.000
	225	0.000	0.015	0.019	0.058	0.000	0.000	0.000	0.000	0.000	0.217	0.111	0.125	0.045	0.023	0.024	0.038	0.000
	227	0.197	0.265	0.096	0.048	0.052	0.056	0.014	0.017	0.000	0.183	0.037	0.000	0.114	0.136	0.095	0.162	0.053
	229	0.000	0.000	0.000	0.038	0.000	0.000	0.027	0.167	0.478	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.026
	231	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.092

Appendix (*contd.*)

Locus	Allele (bp)	Population																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	233	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
	239	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sat7	177	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.078	0.000
	179	0.000	0.000	0.040	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
	181	0.000	0.000	0.050	0.019	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000
	183	0.392	0.394	0.150	0.083	0.500	0.056	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.026	0.031	0.000
	185	0.297	0.242	0.400	0.380	0.086	0.222	0.487	0.371	0.370	0.317	0.037	0.000	0.095	0.265	0.368	0.281	0.257
	187	0.108	0.000	0.020	0.093	0.000	0.000	0.026	0.065	0.043	0.067	0.000	0.000	0.000	0.147	0.079	0.063	0.029
	189	0.135	0.076	0.030	0.046	0.190	0.000	0.026	0.016	0.022	0.067	0.000	0.000	0.000	0.000	0.026	0.000	0.100
	191	0.027	0.227	0.080	0.130	0.138	0.722	0.064	0.161	0.457	0.450	0.037	0.000	0.000	0.324	0.289	0.063	0.557
	193	0.041	0.000	0.200	0.194	0.000	0.000	0.372	0.323	0.087	0.033	0.444	0.342	0.405	0.206	0.132	0.344	0.043
	195	0.000	0.061	0.020	0.019	0.086	0.000	0.000	0.065	0.022	0.050	0.037	0.105	0.024	0.000	0.079	0.016	0.000
	197	0.000	0.000	0.010	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.059	0.000	0.125	0.000
	201	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.105	0.071	0.000	0.000	0.000	0.000
	203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000
	205	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.370	0.421	0.262	0.000	0.000	0.000	0.000
	209	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.024	0.000	0.000	0.000	0.000
	211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000
sat8	134	0.256	0.206	0.148	0.093	0.224	0.222	0.218	0.446	0.000	0.018	0.054	0.105	0.000	0.024	0.125	0.200	0.250
	136	0.000	0.000	0.019	0.185	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.063	0.050	0.056
	138	0.705	0.721	0.833	0.694	0.776	0.722	0.744	0.536	1.000	0.946	0.661	0.895	0.952	0.952	0.813	0.613	0.556
	140	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	154	0.013	0.029	0.000	0.000	0.000	0.056	0.026	0.018	0.000	0.018	0.286	0.000	0.048	0.000	0.000	0.138	0.139
	156	0.013	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	158	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	182	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000