

Population genetic structure and distribution of introduced American mink (*Mustela vison*) in Spain, based on microsatellite variation

Roberta Lecis · A. Ferrando · J. Ruiz-Olmo ·
S. Mañas · X. Domingo-Roura

Received: 5 December 2006 / Accepted: 13 September 2007 / Published online: 29 September 2007
© Springer Science+Business Media B.V. 2007

Abstract The population genetic structure of an invasive species in Spain, the American mink (*Mustela vison*), was investigated using microsatellite DNA markers. This semi-aquatic carnivore, originating from North America, was imported into Europe for fur farming since the beginning of the 20th century. Due to massive escapes, farm damages, deliberate releases and/or accidents, feral mink populations were established in the aquatic ecosystems of many European countries, including Spain. We genotyped 155 American mink originating from the Spanish regions Basque Country, Catalonia, Castilla-Leon, Aragon, Valencia and Galicia using 10 polymorphic microsatellite loci to highlight population genetic structure, distribution and dispersal. *M. vison* populations in Spain appear differentiated and not yet connected by gene flow. Bayesian clustering analyses and spatial analyses of molecular variance detected four inferred clusters, overall coinciding with the sampled geographical localities. Preliminary testing shows moderate to large estimated effective population sizes. Molecular analyses result useful to provide baseline data for further research on the evolution

of invasive mink populations, as well as support local management strategies and indirectly benefit the conservation of threatened species in Spain, such as the endangered European mink (*Mustela lutreola*), and the polecat (*Mustela putorius*), which share the habitat with the American mink.

Keywords *Mustela vison* · *Mustela lutreola* · Invasive species · Population structure · Mink management

Introduction

Alien invasive species are considered today one of the main threats to biodiversity, globally and locally (Sakai et al. 2001; Park 2004). Habitat and ecological changes and the decline or extinction of native species are among the most important consequences of successful introductions/invasions. Few examples exist of recent studies on the population structure and dispersal of invasive alien mammal species, despite the essential role played by understanding the dynamics of populations in order to manage introduced and/or expanding species (Hampton et al. 2004; Abdelkrim et al. 2005). A control program will only be feasible and effective if the spatial structure, the dispersal patterns and the population genetics of the species of concern will be understood and taken into account in any management strategy (Travis and Park 2004). A molecular-based approach can be extremely useful to support management decisions, by highlighting fundamental keys and producing essential information on the bio-invasion process (Holland 2000).

The American mink (*Mustela vison*), a semi-aquatic carnivore originating from North America, was first introduced in Europe at the beginning of the 20th century for fur farming. Due to massive or continual individual escapes,

This paper is dedicated to the memory of Xavier Domingo-Roura.

R. Lecis (✉) · A. Ferrando · X. Domingo-Roura
Institut de Recerca i Tecnologia Agroalimentàries (IRTA),
Genètica de la Conservació, Carretera de Cabrils km2,
08348 Cabrils, Barcelona, Spain
e-mail: roby112@yahoo.com

Present Address:

R. Lecis
Via Cagna No. 66, 09100 Cagliari, Italy

J. Ruiz-Olmo · S. Mañas
Direcció General del Medi natural, Generalitat de Catalunya,
Dr. Roux 80, 08017 Barcelona, Spain

farm damages also caused by hurricanes and fires, deliberate releases and/or accidents, feral mink populations were established in the aquatic ecosystems of many European countries. Feral mink populations are now present in Ireland, UK, Sweden, Denmark, Finland, Netherlands, France, Germany, Austria, Italy and Spain (Palazon and Ruiz-Olmo 1997; MacDonald and Strachan 1999), as well as in Argentina and Chile (Previtali et al. 1998) and in the former Soviet Union. In Spain, the first farms opened during the 1950s in Galicia and Castilla-Leon, followed by others all over the north and centre of the country. Nowadays, the species is represented by wild populations in the northwest (Galicia), the northeast (Basque Country), the centre (Castilla-Leon, Madrid) and the eastern parts of Spain (Catalonia, Aragon and Valencia) (Ruiz-Olmo et al. 1997). During the last few decades, these populations have been expanding their ranges and increasing the number of nuclei in almost each colonized area (Bravo and Bueno 1992; Ruiz-Olmo et al. 1997). Populations might have increased today to three or four nuclei in Galicia, two or three in Basque Country, one in central Spain, in Catalonia and in Teruel-Castellon (Invasiber, <http://www.hidra.udg.es/invasiber/presentacion.php>). Although the number of mink farms in Spain was dramatically reduced at the end of the 1980s, massive mink escapes from the last active farms are still ongoing (i.e., in Galicia, June 2005, R. Romero pers. comm.).

Introduced *M. vison* can represent a threat to native species such as the endangered and declining European mink (*M. lutreola*), today represented by one last isolated population in the north of Spain, and the polecat (*M. putorius*). American mink may act as an ecological competitor and also as a disease vector of Aleutian mink parvovirus (Mañas et al. 2001; Fournier et al. 2004). Introduced mink, adaptable and opportunistic in their diet, can also affect various prey species, such as native crustaceans, fish, amphibians, water voles and waterfowls (Ferrerias and MacDonald 1999; Rushton et al. 2000; Nordstrom et al. 2003; Delibes et al. 2004). In Spain, *M. vison* can prey on protected species such as the native freshwater crayfish (*Austropotamobius pallipes*) and the Iberian desman (*Galemys pyrenaicus*), also on water voles (*Arvicola sapidus*), fish (*Gasterosteus aculeatus*) and amphibians (*Euproctus asper*), as well as impact on human activities such as fish management and sport fishing (Palazon and Ruiz-Olmo 1997). Various studies have been conducted on the predatory impact of American mink on different species (Craik 1997; Clode and MacDonald 2002) and on the ecological interactions among *M. vison* and other mustelidae (especially *M. lutreola* and the otter *Lutra lutra*: Sidorovich et al. 1999; Sidorovich and MacDonald 2001; Bonesi and MacDonald 2004a, b, Bonesi et al. 2004).

Studies on the coexistence between otters and mink (Bonesi and MacDonald 2004a) showed that otter presence is associated with significant and rapid reduction in mink densities, and the expansion rate of mink populations tends to be higher in areas of Spain where the otter and the polecat are not present (Ruiz-Olmo et al. 1997). The development of population control strategy of American mink in Europe is a controversial issue which has been faced in different ways by different countries. In Spain, a control program is running since 1999, within LIFE projects linked to the conservation of the European mink.

While the effects of invasive species are usually well studied, the changes in population genetic structure and dynamics of such species during the bio-invasion process are rarely investigated using contemporary molecular techniques and not always understood (Bryan et al. 2005; Hampton et al. 2004). Genetic studies based on mitochondrial DNA sequences have been conducted to identify mustelid species by analysis of DNA extracted from faecal samples (Hansen and Jacobsen 1999; Gomez-Moliner et al. 2004), and to highlight the phylogeography of the European mink (Davison et al. 2000), while microsatellite genotyping has been used to survey genetic diversity and population history of *M. lutreola* populations (Peltier and Lodé 2003; Michaux et al. 2004, 2005), of other mustelidae species (*M. nigripes*, *M. eversmanni*, *M. putorius*: Wisely et al. 2002) and to investigate variability in captive *M. vison* (Belliveau et al. 1999). A rich library of microsatellite loci exists on *M. vison* (O'Connell et al. 1996; Brusgaard et al. 1998a–c, Davis and Strobeck 1998; Fleming et al. 1999; Vincent et al. 2003; Anistoroaei et al. 2006), although not all loci have proved to be polymorphic and informative or have been tested on populations. Up to now, genetic information on feral American mink populations introduced in Europe is scarce or completely lacking. Considering that the invasion of European ecosystems by this mustelid is a recent process, a genetic analysis should take into account this time frame in the choice of molecular markers and the captive history of these feral populations in the discussion of the results. On the other hand, it is essential to provide baseline data on such an invasive already common mammal species at an early stage of its population processes, in order to help understanding colonization patterns in the future.

In this study, we investigate microsatellite loci variation in American mink populations in Spain in order to (i) elucidate their genetic structure, (ii) evaluate dispersal patterns and give preliminary data on the presence of gene flow and/or isolation among populations, (iii) support management strategies and indirectly improve the conservation of rare or endangered mustelids in Spain.

Methods

Sample collection

A total of 155 tissue samples were collected from free ranging American mink trapped between 2001 and 2004 in Alava, Basque Country (30), Barcelona and Girona, Catalonia (32), Burgos and Palencia, Castilla-Leon (30), Teruel, Aragon (30) and Castellon, Valencia (28), Pontevedra, Galicia (5) (Fig. 1). Tissue samples were collected during control operations conducted by local administration as part of an eradication program, and stored in 95% ethanol. Basic demographic (age, sex, weight, date and site of collection) were also collected for each animal. Samples originating from Teruel and Castellon were pooled in one population (Aragon + Valencia) for all subsequent analyses, because of their geographical proximity.

DNA extraction, PCR amplification and microsatellite scoring

Total DNA was extracted using Dneasy Tissue kit (Qiagen), leaving the digestion in Proteinase K overnight and following the protocols for animal tissue. Ten polymorphic microsatellite loci which have been isolated in Mustelidae species (O'Connell et al. 1996; Fleming et al. 1999) have been selected to genotype each sampled mink (Table 1). Some of these loci already proved informative in *M. lutreola* (Peltier and Lodé 2003) and captive *M. vison* (Belliveau et al. 1999) genetic diversity and variability studies. PCR amplification of microsatellites was performed as follows: first denaturation at 95°C for 1 min,



Fig. 1 *Mustela vison* distribution and sampled areas. Within the shaded areas, indicating American mink distribution in Spain, the numbers of samples used in this study

30 cycles of denaturation at 94°C (20 s), annealing at 55–58°C (20 s) and extension at 74°C (20 s), followed by a final extension at 72°C ranging from 10 to 30 min. PCR reactions were performed in 10 µl volumes containing 0.3 µM each of forward (labelled with Hex, Fam or Ned fluorescent dyes) and reverse primers, 1X Taq buffer, 0.5 units of EcoTaq DNA polymerase, 200 µM of dNTPs, 2 mM MgCl₂ and approximately 40 ng of genomic DNA. Diluted PCR products were pooled in sets of three to four loci and analysed using an ABI 3100-Avant automatic sequencer. Allele size was determined by co-running a size standard (ROX 500) and DNA fragments were scored using the software Genescan 3.7 (ABI).

Analyses of genetic variation

Commonly used population genetic statistics were computed for each locus and population using GENETIX 4.02 (Belkhir et al. 2001), GENEPOP 3.2 (Raymond and Rousset 1995) and FSTAT (Goudet 1995) programs. We analyzed allele frequencies and distribution, expected (H_e) and observed (H_o) heterozygosity values, deviations from Hardy-Weinberg Equilibrium (HWE, through F_{is} values following Weir and Cockerham 1984) and Linkage Disequilibrium for all populations, with adjusted P -values for 1% nominal level based on 4,500 permutations. Bonferroni corrections for multiple comparisons were used to find critical significance levels for both tests (equivalent to $P < 0.05$). We investigated differences in allelic richness (number of alleles independent of sample size) among populations using FSTAT. Sex biased in dispersal was tested among American mink populations investigating assignment index AI, its mean and variance, F_{st} , F_{is} and H_s (within group gene diversity) values, using the program FSTAT, two-sided tests and 1,000 randomisations, as described by Goudet et al. 2002.

Population structure analyses

Pairwise F -statistics was evaluated to estimate the extent of inter-population distances through analysis of molecular variance (AMOVA) as in ARLEQUIN 2.0b2 (Schneider et al. 2000) and through pairwise mean F_{st} (Weir and Cockerham 1984) and R_{st} (Rousset 1996) as in GENEPOP 3.2. GENETIX was used to visualize patterns of population differentiation by Factorial Correspondence Analysis (Benzecri 1973) of individual multilocus genotypes.

Bayesian clustering and assignment tests implemented in STRUCTURE 2.1 (Pritchard et al. 2000; Falush et al. 2003) were used to analyze the population structure of our mink samples and to assign individuals to their likely

Table 1 List of microsatellite loci used to genotype American mink (*Mustela vison*) populations in Spain, with their annealing temperatures (T_a , °C), repeat type and number, primer sequences and source

Locus	T_a (°C)	Repeat	F primer sequence	R primer sequence	Source
Mvi57	58	GT(17)	GAACAGGACCAGCCCTGC	GTTGGAAATGAGGATCTCAC	O'Connell et al. (1996)
Mvi87	58	GT(16)	AAAATAGTAGTGGCAGCAGC	GTCTGTGAAACACTGCAAAGC	O'Connell et al. (1996)
Mvi111	55	GT(15)	GTGGGCATAGAATTTAGAGG	TTATCAAAGACAATGTGCGAG	O'Connell et al. (1996)
Mvi114	55	GT(12)	TTGTAAGAGTTAGAGGTAATC	AAACTCAAAATTGTCCTAAGCC	O'Connell et al. (1996)
Mvi219	55	GT(-)	GGGTGCGGCTCTCACTGC	GGAGTATTGTCCTCACCTGC	O'Connell et al. (1996)
Mvi232	55	GT(13)	GACGATTCACAAACCTATAACC	TCACCAGGGACCAACAGGT	O'Connell et al. (1996)
Mvis002	55	CA(11)	TGGGAAAAATAGTGCTCCAAAG	AAACAGCAGAGAGCATAACAGCC	Fleming et al. (1999)
Mvis022	57	CA(13)	ATCAAGTCCTGCATCAGGCT	TGGGCTGTTTGTCCAGGT	Fleming et al. (1999)
Mvis075	55	CA(12)	GAAATTTGGGGAATGCACTC	GGCAGGATAGGATGTGAGCT	Fleming et al. (1999)
Mvis099	57	CA(16)	TGAGGCAAGAGGAGCAAAAAG	TTTGCATTTCCCTGATGAGG	Fleming et al. (1999)

population of origin. This model-based method is designed to infer the most likely number of K populations (genetic clusters) found in a set of sampled multilocus genotypes, and compute the proportion of membership of each pre-defined (geographical) sample group and of each individual genotype to the inferred clusters. In this study we used the admixture model to analyse the total sample set of 155 mink originating from different areas in Spain, comparing the outputs of the I model (assuming independent allele frequencies) and the F model (allele frequencies correlated). Our results are based on simulations from 1 to 10 ($K = 1-10$) inferred populations, running STRUCTURE with 5 repetitions of 10^5 iterations of a Markov chain Monte Carlo, following a burn-in period of 10^4 iterations. A threshold of $q > 0.80$ was used for the assignment of individual genotypes to one cluster (or jointly to more clusters, if the proportion of membership to each one was $q < 0.80$).

Spatial and population size analyses

Evidence of recent effective population size reductions was investigated using the program BOTTLENECK 1.2 (Cornuet and Luikart 1996), which tests for an excess in heterozygosity as a consequence of a genetic bottleneck, following Luikart and Cornuet 1998. Microsatellite data were tested under a 100% stepwise mutation model (SMM, Kimura and Ohta 1978) and a two-phase mutation model (TPM with 70% SMM, Di Rienzo et al. 1994), assessing significance by the Sign and the Wilcoxon sign-rank test and also testing allele frequency distributions for shifts from the equilibrium L-shape (Luikart et al. 1998). We used BAYESASS (Wilson and Rannala 2003) to estimate recent migration rates (m) between mink populations and also to provide individual assignments to each population, classifying migrants, no-migrants and offspring of migrants. NeESTIMATOR (Peel et al. 2004) was used to estimate effective population sizes (N_e) from allele

frequency data for each *M. vison* population, using two point estimation methods, the linkage/gametic disequilibrium-based one (Hill 1981) and the heterozygote excess-based one (Pudovkin et al. 1996).

We explored how much differentiation between the five mink populations might be explained by geographical distance (isolation by distance) using the subprogram ISOLDE implemented in GENEPOP. Spatial Analysis of Molecular variance was performed using SAMOVA 1.0 (Dupanloup et al. 2002), to define populations which are apparently geographically homogeneous but genetically differentiated from each other, and to identify possible genetic barriers between the groups. We assumed the geographical coordinates of Alava, Barcelona, Burgos, Castellon and Pontevedra as generally defining the spatial structure of our mink populations from Basque Country, Catalonia, Castilla-Leon, Aragon + Valencia and Galicia respectively. SAMOVA was run with K (Number of groups) ranging from 2 to 4, Number of initial conditions (number of simulated annealing processes to perform) = 100 and Sum of squared size difference as the considered molecular distance between haplotypes. We also analyzed our data with the package GENELAND (Guillot et al. 2005), in order to further investigate the number of populations and the spatial location of genetic discontinuities between them, at first allowing K to vary (1 to 10), 100,000 MCMC iterations, uncertainty attached to spatial coordinates fixed to 1 km, and then with the fixed modal K obtained and the other parameters unchanged (as in Coulon et al. 2006).

Results

Genetic diversity of mink populations

All loci were polymorphic in the 155 genotyped American mink, except locus Mvis002, which resulted monomorphic

Table 2 Summary of allelic variation at 10 microsatellite loci used to genotype American mink populations (*M. vison*) in Spain

Locus	Allele size range	Number of alleles	F_{is} BC	F_{is} CA	F_{is} CL	F_{is} A + V	F_{is} G
Mvi57	92–102	6	0.036	0.007	0.062	0.014	0.238
Mvi87	77–83	4	0.620*	0.659	–	0.438*	0.529
Mvi111	86–106	6	0.213	–0.131	–0.109	0.048	–0.290
Mvi114	63–83	7	0.122	0.008	–0.064	–0.134	–0.212
Mvi219	162–176	6	0.009	0.048	–0.035	0.074	–0.067
Mvi232	142–158	7	–0.153	0.205	–0.087	–0.061	0.059
Mvis002	175–185	4	0.227	–0.041	–0.115	–0.018	–
Mvis022	279–285	4	–0.287	0.071	–0.102	0.169	0.040
Mvis075	114–128	8	0.052	0.405	0.108	–0.072	0.250
Mvis099	320–344	9	0.249	0.464*	0.189	0.156	0.360
			BC	CA	CL	A + V	G
Average F_{is}			0.099	0.137	–0.007	0.062	0.092
Average H_e			0.6137	0.6034	0.5944	0.6231	0.6080
Average H_o			0.5673	0.5306	0.6090	0.5897	0.6200

BC, Basque Country, CA, Catalonia, CL, Castilla-Leon, A + V, Aragon + Valencia, G, Galicia

For each locus, microsatellite ID, allele size range and number of alleles found in our samples are listed. F_{is} following Weir and Cockerham (1984) was calculated for each locus and each population using the Hardy Weinberg exact test, significant F_{is} value (at a probability level equivalent to $P < 0.05$ after Bonferroni correction for multiple comparisons) are indicated by an *. In the second part of the table, average values of F_{is} , and average values of: expected heterozygosity (H_e) and observed heterozygosity (H_o) for each population

in samples originating from Teruel (Aragon), fixed for the allele 185 (however, this microsatellite showed quite limited variation in all cases, with a maximum of three alleles per population). All analysed loci were moderately variable, showing between 4 and 9 alleles per locus, and we detected private alleles in all populations (3 in Basque Country, 2 in Aragon + Valencia, and 1 in Catalonia, Castilla-Leon and Galicia populations respectively). Allelic diversity ranged from 3.9 alleles on average in samples from Galicia, to 4.7 alleles on average in samples from Aragon + Valencia. Allelic richness was found higher in loci Mvis075 and Mvis099, and Basque Country and Aragon + Valencia populations. Table 2 shows a summary of allelic variation at the 10 microsatellite loci analysed, as well as F_{is} and heterozygosity values per locus and per population. Expected heterozygosity ranged from 0.5944 (Castilla-Leon) to 0.6231 (Aragon + Valencia), and observed heterozygosity was lower than expected in three cases, while resulted higher in the other two populations. Performing Hardy–Weinberg exact test by locus and population and applying Bonferroni correction for multiple comparisons, only 3 values of F_{is} were found significant; however, the average F_{is} values across loci were not significant. All loci appear in genetic equilibrium considering all populations, using a P -value adjusted for 1% nominal level and 4,500 permutations, except loci Mvis002 and Mvis099. There was no evidence of significant sex biased dispersal analysing our data (results not shown). Indeed, following the hypothesis that *M. vison* males disperse more than females (Peeples et al. 2002), F_{st} values were higher

and F_{is} values lower for the more phylopatric sex, and H_s value (within group gene diversity) was slightly larger for the group dispersing most. However, none of the P -value associated to the tests were significant ($P < 0.05$).

Population structure and variation

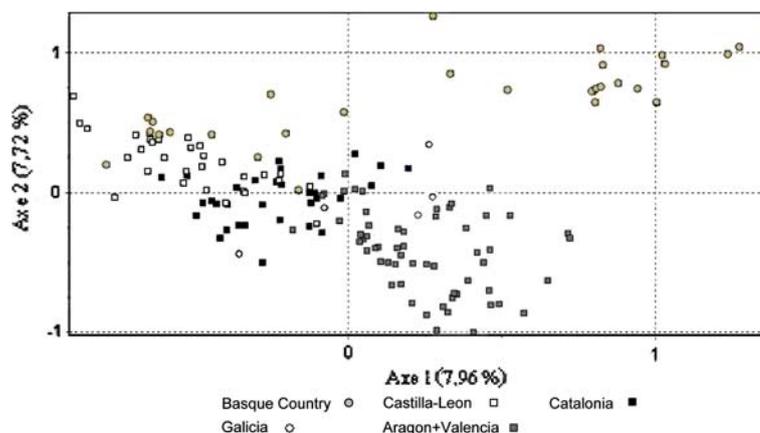
F -statistics revealed significant population differentiation for most pairwise comparisons, especially between the mink populations from Basque Country and Catalonia (highest $F_{st} = 0.148$), while the lowest F_{st} value (0.049) is found between Aragon + Valencia and Galicia samples (Table 3). R_{st} values overall replicate the F_{st} pattern for the highest and lowest values, although showing more variability in all intermediate population comparisons. The scatter plot obtained performing Factorial Correspondence Analysis (FCA) shows individuals from Basque Country population scattered over the graph revealing some degree of intra-population variability (Fig 2). Individual mink genotypes sampled in each of the other localities (Catalonia, Castilla-Leon, Aragon + Valencia) group together in the graph, although showing some degree of population overlapping. Especially mink genotypes from Aragon + Valencia and Galicia spatially overlap, as expected after looking at F_{st} results (see Fig 2). Extrapolating results on Galicia mink population should be done cautiously due to the small sample size considered.

The program STRUCTURE was run using the admixture model and alternatively the I (independent allele

Table 3 Population pairwise R_{st} (above diagonal) and F_{st} (below diagonal) estimates of differentiation among five feral mink populations, as computed by GENEPOP (Weir and Cockerham 1984; Rousset 1996)

	BC	CA	CL	A + V	G
BC	–	0.238	0.137	0.067	0.056
CA	0.148	–	0.096	0.169	0.098
CL	0.122	0.105	–	0.098	0.070
A + V	0.112	0.123	0.112	–	0.035
G	0.074	0.134	0.128	0.049	–

BC, Basque Country; CA, Catalonia; CL, Castilla-Leon; A + V, Aragon + Valencia; G, Galicia

Fig. 2 Factorial Correspondence Analysis of individual American mink multilocus genotypes from 5 populations in Spain, computed using 10 microsatellite loci

frequencies) and F model (allele frequencies correlated), and the output did not substantially change. Considering that the genotyped mink originally came from ‘independent’ farms situated in different regions in Spain, the I model should be in principle the most appropriate to treat our data. However, not knowing the degree of animal trade and interchange among fur farms in Spain, or the possible shared ancestry of farmed mink, and wishing to maximize clustering for closely related populations (Falush et al. 2003), we also considered the F model as appropriate.

Running Bayesian analyses under the admixture and F model, we obtained two comparable outputs detecting respectively 4 (a) or 5 (b) discrete inferred populations (Table 4, Fig 3). Both models were associated to very similar values of likelihood: Estimated Ln prob of data: $\text{LnP(D)}_a = -3724.2$; $\text{LnP(D)}_b = -3672.1$. Considering $K = 5$, Galicia samples come out as a distinct cluster (cluster 2, with proportion of membership $Q = 0.766$), while with $K = 4$, they result admixed between clusters 1 ($Q = 0.353$) and 3 ($Q = 0.334$), with marginal contribution from the other two clusters as well. Bayesian analyses show that LnP(D) values increased sharply with K from 1 to 4, then reaching a fluctuating plateau (Fig 4). Applying the formula $(\text{LnP(D)}_k - \text{LnP(D)}_{k-1})$ (Garnier et al. 2004), apparently the optimal subdivision of the data is given by splitting the samples in 4 clusters, avoiding unjustified oversplitting (Fig 4), consequently model a) ($K = 4$) was

given priority in the further elaboration and discussion of results. Excluding Galicia, each geographical population corresponds to a genetic cluster, with the exception of Basque Country mink, split between cluster 3 ($Q = 0.432$) and 4 ($Q = 0.534$) (Fig 3, Table 4). Mink from Catalonia group in cluster 2, with proportion of membership $Q = 0.948$, while Castilla-Leon individuals fall into cluster 3 ($Q = 0.895$) and Aragon + Valencia animals into cluster 1 ($Q = 0.832$).

There is wide correspondence among the individual assignment results obtained with STRUCTURE and BAYESASS: all mink apparently cluster similarly. Especially in the case of Basque Country population, where both Bayesian analyses identify two genetic clusters, there is high correspondence between clusters 3 and 4 in the STRUCTURE output (Table 4) and populations 1 and 3 in the BAYESASS output (data not shown). The migration rate mean and 95% confidence interval calculated by BAYESASS for data sets with the five populations are 0.0417 (1.79E–05, 0.185). Values of migration rates into each populations are overall quite low (ranging from 0.001 to 0.13).

Bottleneck effects and Effective population size

Table 5 shows BOTTLENECK results, used to test whether *M. vison* populations in Spain showed excess

Table 4 STRUCTURE results (using admixture model, allele frequencies correlated). Proportion of membership of each geographically defined American mink population in each of the 4 (a) or 5 (b) inferred ($K = 4, K = 5$) genetic clusters. Values in bold indicate the most likely cluster(s) of origin for each population

Pop	Inferred clusters				<i>N</i> samples	
	1	2	3	4		
<i>(a) LnP(D) = -3724.2</i>						
BC	0.016	0.018	0.432	0.534	30	
CA	0.019	0.948	0.024	0.009	32	
CL	0.035	0.063	0.895	0.008	30	
A + V	0.832	0.086	0.064	0.018	58	
G	0.353	0.150	0.334	0.163	5	
Pop	Inferred clusters					<i>N</i> samples
	1	2	3	4	5	
<i>(b) LnP(D) = -3672.1</i>						
BC	0.404	0.048	0.528	0.010	0.009	30
CA	0.016	0.144	0.008	0.819	0.014	32
CL	0.814	0.113	0.006	0.049	0.017	30
A + V	0.013	0.233	0.011	0.023	0.719	58
G	0.047	0.766	0.100	0.019	0.068	5

BC, Basque Country; CA, Catalonia; CL, Castilla-Leon; A + V, Aragon + Valencia; G, Galicia

Fig. 3 Bar plots obtained from STRUCTURE, analysing the population structure of American mink in Spain, with $K = 4$ (above) and $K = 5$ (below) inferred clusters. Each individual is represented by a vertical line partitioned into K coloured segments. 1 = Basque Country (30), 2 = Catalonia (32), 3 = Castilla-Leon (30), 4 = Aragon (30) + Valencia (28), 5 = Galicia (5)

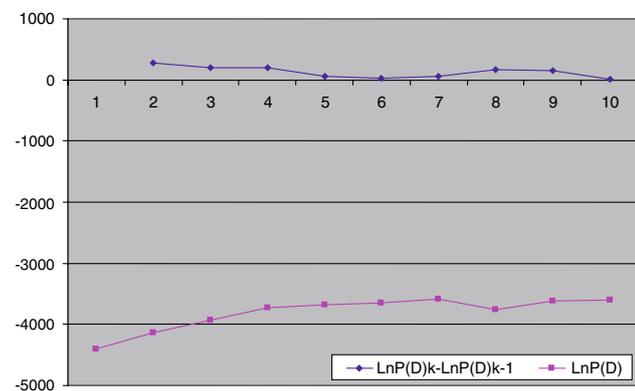
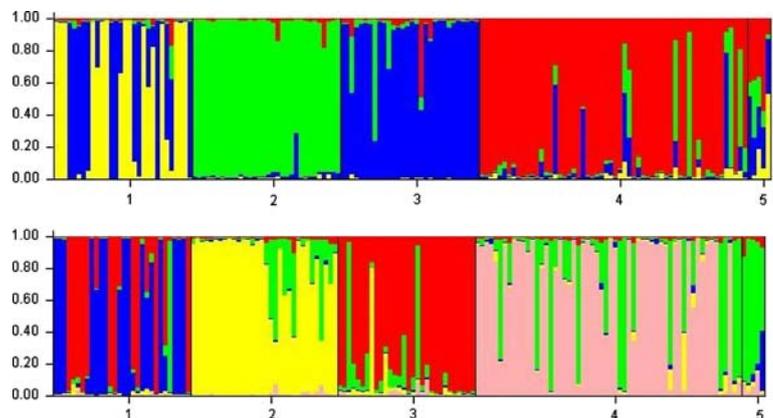


Fig. 4 Posterior probability of the data $LnP(D)$ against the number of K clusters, and increase of $LnP(D)$ given K , calculated as $(LnP(D))_k - LnP(D)_{k-1}$

heterozygosity, as expected after a recent bottleneck. There was evidence of bottleneck effects in the Catalonia population, which showed significant P -values in both Sign and Wilcoxon tests under the TPM model of mutation (70% SMM, $P < 0.05$), and also a shifted mode in the distribution of allele frequencies. The TPM model is thought to be the more realistic model when analyzing microsatellite data (Cornuet and Luikart 1996). Aragon + Valencia population showed a significant heterozygosity excess under the TPM, performing both tests ($P < 0.05$), but a normal L-shaped distribution of allele frequencies. Apparently only the American mink population in Catalonia was affected by a bottleneck, experiencing a severe reduction in number, due to founder effect (linked to farm escapes origin) or eradication control measures, despite retaining a considerable

Table 5 Test for the presence of recent bottleneck in American mink populations in Spain. *P* values are reported, a significance level of $P < 0.05$ is indicated by an *

TEST	BC	CA	CL	A + V
<i>Sign test</i>				
100% SMM	0.374	0.334	0.120	0.332
70% SMM	0.146	0.031*	0.118	0.030*
<i>Wilcoxon test</i>				
100% SMM	0.492	0.032*	0.131	0.322
70% SMM	0.232	0.005*	0.105	0.005*
Mode shift	Normal L-shaped	Shifted	Normal L-shaped	Normal L-shaped

BC, Basque Country; CA, Catalonia; CL, Castilla-Leon; A + V, Aragon + Valencia

Galicia results are not shown due to the program warning that the effective of the population may be too low to obtain a reliable output

level of genetic diversity ($H_e = 0.6034$, but $H_o = 0.5306$, Table 2), see the Discussion section for details.

Table 6 shows results obtained using the software NeESTIMATOR, implemented to estimate effective population size (N_e) from allele frequency data (Peel et al. 2004). Using the linkage/gametic disequilibrium method (Hill 1981) we obtained values of N_e ranging from 7.2 (Basque Country and Galicia) to 34.8 (Aragon + Valencia). Performing the heterozygote excess point estimation method (Pudovkin et al. 1996), almost all sampled mink populations resulted large and abundant. ‘Infinity’ means that a finite result was not possible with the current data and the method could not distinguish between large and infinity (consequently not giving a finite 95% confidence interval).

Relating genetic structure to geographic distances

No isolation by distance effect was found in mink populations when performing the subprogram ISOLDE implemented in GENEPOP, which computes a regression of $F_{st}/(1-F_{st})$ estimates to the logarithm of geographic

distances: the test of isolation by distance gives a non significant *P* value (0.1764 under null hypothesis). The Spatial Analysis of Molecular variance defined four groups of populations ($K = 4$) maximally differentiated from each other and associated to the highest *F*ct fixation index (0.08974), which is the proportion of total genetic variance due to differences between groups of populations (Dupanloup et al. 2002), therefore giving an output consistent to the one obtained running STRUCTURE. SAMOVA grouped Aragon + Valencia and Galicia populations together (as in FCA analysis, Fig. 2) and then individually Basque Country, Catalonia and Castilla-Leon. On the other hand, GENELAND results (4 outputs out of 5 runs) show the presence of five populations in our multilocus data, supporting the existence of a fifth cluster corresponding to Galicia (data not shown), and confirming the distribution of the other 4 clusters already identified by STRUCTURE.

Discussion

General summary of results

The successful establishment and spreading of introduced species in novel environments depend on a number of different ecological and genetic factors. There are examples of successful invasive species characterized by low genetic diversity and extreme loss of molecular variation (Grapputo et al. 2005; Lindholm et al. 2005; Pinceel et al. 2005) although high genetic variability, large number of founders and multiple founding sources usually significantly contribute to an alien species’ introduction success (Barbaresi et al. 2003; Johnson and Starks 2004; Stepien et al. 2005).

In this study, we found a moderate to high degree of genetic variation in feral *M. vison* populations sampled in Spain, with heterozygosity values ranging from 0.5306 to 0.6200 (H_o) (Table 2). Belliveau et al. (1999), genotyping captive and wild individuals, found average heterozygosity

Table 6 NeEstimator results on American mink populations in Spain, using the linkage disequilibrium (LD) and the heterozygote excess (HE) point estimation methods

Pop	Estimate (LD)	Estimate (HE)
BC	7.2 (6.2–8.4)	Inf
CA	23.2 (16.9–34)	Inf
CL	22.2 (16.6–31.4)	58.3
A + V	34.8 (27.5–45.3)	Inf
G	7.2 (4.5–15.2)	Inf
All populations	33.4 (29.8–37.4)	Inf

BC, Basque Country; CA, Catalonia; CL, Castilla-Leon; A + V, Aragon + Valencia; G, Galicia

Estimate: effective population size estimate (Peel et al. 2004). Inf—Infinity. Values in brackets indicate the approximate 95% CI (Confidence Interval)

values of 0.498 (H_e) and 0.370 (H_o) in the wild mink population (although their sample may not be representative of the Canadian wild mink population). On the basis of the observed genotypes, at least four of the mink populations analysed are genetically differentiated. This is consistent with their origins from different farms all over Spain and with the typical mink distribution, which establish populations along river basins occupying linear territories spanning 1–6 km (Dunstone 1993). Overall, American mink populations in Spain appear quite healthy, when considering both their molecular diversity and some of their estimated effective population sizes (Table 6). Genetically speaking, there could be an overestimation of both genetic diversity and population sizes, reflecting the different origins of the founders of captive populations. However, ecological studies suggest that mink population sizes might actually be larger than those inferred by our analyses (Ruiz-Olmo pers. comm.). Results also confirm the ecological adaptability of the species to different environments (such as those present in Spain), a wide prey range, new habitats and locations (Dunstone 1993).

Assumptions and limits

Despite the captive and recent origins of the analysed feral mink populations, all of them aged not more than five decades (the first fur farm in Spain opened in Segovia, Castilla-Leon, in 1958, Palazon and Ruiz Olmo 1997), we could detect a clear population structure when analysing microsatellite genotypes of sampled mink. The historical farm distribution and the genetic selection operated on mink stocks originating from different ranches likely affect these data and this is taken into account in our discussion. Unfortunately, there are not detailed historical records of the distribution of mink farms, the degree of mink trade between them, nor on the history of releases/escapes, apart from general information (Palazon and Ruiz Olmo 1997; Ruiz-Olmo et al. 1997). Given American mink high reproduction rates and the variability of the selected molecular markers, we attempted not to merely detect the captive history of mink stocks in Spain, but start highlighting actual trends in population diversity and structuring, considering the potential influence of both historical and contemporary processes. Examples of molecular studies on recently established wild populations of invasive species already exist (Barbaresi et al. 2003; Zeisset and Beebe 2003; Hampton et al. 2004; Lindholm et al. 2005). All results on Galicia population are considered cautiously, due to the small sample size available. The existence of a potential fifth genetic cluster identified by Geneland and one of Structure outputs (see Fig. 3, $K = 5$; Table 4, b) should be tested performing further analyses including more Galician samples.

Population structure and individual assignments

Bayesian analyses identify in our multilocus genotype dataset four genetic clusters corresponding to Aragon + Valencia (cluster 1), Catalonia (cluster 2), Castilla-Leon and Basque Country I (cluster 3), Basque Country II (cluster 4). Clusters 1 and 3 also contribute to define Galicia samples. Apart from Basque Country mink population, split into 2 clusters, the Q values are generally high and >0.80 (our threshold value), indicating a good resolution of the data (Table 4a). The overlapping distribution of genotypes coming from Castilla-Leon and Basque Country I might suggest some degree of gene flow existing between the two populations, which would be possible due to the geographical proximity of the regions. However, this result is not particularly supported by other analyses (i.e., F_{st} and SAMOVA), and there are other possible explanations: for instance, Castilla-Leon and part of the Basque Country populations might originate from similar farmed mink stocks, which retain traces in the actual genotypes of free-ranging mink in Alava.

There is high correspondence between individual results obtained with the Bayesian clustering methods implemented in STRUCTURE and BAYESASS. In the case of Basque Country population, both analyses indicated the presence of two genetic clusters within the samples (Fig 3, Table 4). Relating detailed geographical origins to cluster assignments for each mink sampled in the Alava area, we could not detect any particular pattern explaining the genetic distribution of individuals (i.e. mink falling into a cluster all coming from the same locality). Apparently the two genetic components of Basque Country population are not yet spatially defined or, as mentioned above, possibly originate from two distinct farm genetic stocks which are currently geographically overlapping.

Genetics related to geography, Dispersal patterns

Predicting mink distribution in Spain plays an essential role in planning a strategy for managing its impact on native wildlife. However, building statistical models to predict species occurrence is not a simple task, due to the high adaptability of the species and the changing nature of its actual distribution (MacDonald and Strachan 1999). An attempt has been done by Muñoz et al. (2005) who applied Generalized Linear Models (GLM) based on mink distribution and on a number of habitat variables in order to predict the expansion of mink populations in Spain. The models identify areas of suitable habitat all around the areas already occupied by *M. vison*, therefore predicting a potential expansion and contact among mink nuclei in the near future. In our study, Bayesian clustering method, SAMOVA and FCA analyses show that *M. vison*

populations are quite differentiated and, although there is no statistical evidence of isolation by distance, apparently not yet connected by current gene flow. However, as mentioned above, ecological (Ruiz-Olmo et al. 1997) and modelling (Muñoz et al. 2005) evidence suggest a possible future expansion, which would be supported by the genetic variation found in populations and the potential adaptability of individuals to different environments. Mink dispersal routes could possibly join the most geographically proximate populations, such as Castilla-Leon and Basque Country in the north of Spain, and Catalonia and Aragon + Valencia in the north-east. The Ebro river basin, connecting Basque Country and Catalonia, also represents a potential dispersal route for mink population expansion and contact.

Bottlenecks and effective population size

Although handling recent feral populations, which might not be in mutation-drift equilibrium, we tested them for recent bottleneck presence and performed preliminary analyses on population size estimates. In this study, a genetic bottleneck could be interpreted as founder effect, i.e. mink population originating from few escaped individuals, or as the reduction of a wild population to low numbers, for example due to recent trapping control measures. Given the captive history, results should be handled with caution. Bottleneck effects could be masked by multiple sources contributing to farm stocks and/or it could be too soon to detect contemporary processes shaping heterozygosity in populations. Apparently, most feral mink populations in Spain (except the Catalonia one) have not suffered any genetic effects of the founder event (Table 5), or these effects were promptly ameliorated by the rapid growth of the newly established populations and/or continual escapes contributing to the genetic stock in the wild. Further research would be useful to compare levels of genetic diversity between native and introduced American mink populations (as done by Grapputo et al. 2005).

NeESTIMATOR results (Table 6) show discordant values when using two different estimation methods. Caution should also be used interpreting these results, as there could be an overestimation of effective population sizes, if founders of feral populations came from different farm stocks and/or farm founders originally came from different wild populations. Performing the heterozygote excess estimation method, the only population resulting comparatively smaller than the others was the Castilla-Leon one, with $N_e = 58.3$ (Table 6). Using the linkage disequilibrium point estimation method, Basque Country and Galicia populations appear the most limited ones

(Estimate: 7.2), in terms of effective population size and probably absolute numbers. This is consistent with management and ecological data available on these *M. vison* nuclei. The Basque Country population has been subjected to intense control measures, as in this area American mink presence represents a direct threat to the last population of European mink. The mink population in Galicia, with at least two nuclei in Pontevedra and La Coruña, has been known to be stable at low densities since years, also because of the otter presence in the area (Palazon and Ruiz Olmo 1997). However, local references suggest that mink populations in Galicia could be currently expanding (R. Romero pers. comm.) and improving the analysed sample size from this area might modify the result. Aragon + Valencia population appears to be the largest (Estimate: 34.8), with an inferred population size of at least approximately 348 free ranging individuals (based on an estimate of long-term effective population size as, on average, 1/10 of actual sizes, Frankham et al. 2002).

Implications for American mink management and conservation of other Mustelidae

The American mink is found today in many European freshwater ecosystems with established and expanding wild populations. Complete eradication of this species seems unrealistic; however, many conservation organizations in Europe consider a reduction in numbers as a short-term solution (Tyler et al. 2005), especially where mink presence represents a direct threat to native species. This happens in the north of Spain, where *M. vison* and the highly endangered *M. lutreola* compete for habitat and preys. The European mink, one of the most threatened carnivores in Europe (<http://www.redlist.org>, Palazon et al. 2002; Michaux et al. 2004, 2005), is represented by one last isolated population in western Europe, inhabiting an area between the north of Spain and the south-west of France. Where their ranges overlap, the American mink compete successfully with the smaller and more specialist European mink, due to its larger body size, more opportunistic habits and larger litter size (Maran and Henttinen 1995; Sidorovich et al. 1999; Sidorovich and MacDonald 2001). Eradication measures and trapping sessions of *M. vison* in Spain, when implemented as a short-term control strategy, should be concentrated in European mink inhabited areas (Basque Country, along the Ebro basin). However, *M. vison* populations are apparently already part of the European fauna, and locally of the Iberian fauna. Wherever in Europe a control program was attempted, it was impossible to eradicate this introduced species, especially for its reproductive capacity and for difficulties in capturing individuals (Palazon and Ruiz-Olmo 1997).

As discussed by Schlaepfer et al. (2005), even when it is feasible to eradicate an invasive species at a given location, likely re-colonization from adjacent areas will occur, rendering efforts and investments in vain. Given these considerations, alternative ways to manage this species should be evaluated, together with attempting to neutralize further sources of introductions: mink farm should be closing down wherever possible and/or high security systems applied. Various management alternatives should be explored, including the effect of reintroducing the otter in areas inhabited by the American mink, as a mean of ecologically controlling mink numbers (Bonesi and MacDonald 2004a). Sterilization and/or immunocontraception of reproductive individuals and the use of exclusion barriers should also be explored without prejudice as possible control strategies. As discussed by Tyler et al. (2005), any management design needs to incorporate experimental and control areas, in order to attribute the decrease in the abundance of *M. vison* at a given locality to the particular control measure applied.

Management programmes of invasive species all over Europe usually proceed in the absence of data regarding the genetic structure of recently established populations, while this information is essential to understand invasion processes and dynamics (Estoup et al. 2001; Bryan et al. 2005). As discussed by Hampton et al. 2004; incorporating molecular-based information into management planning would definitely improve invasive species management strategies, just as it has aided the conservation of endangered species. Our results suggest that farm breeding and multiple escapes all over Spain may have increased genetic variability in feral mink nuclei, creating the conditions for adaptable and expanding populations. Considering the levels of differentiation, scarce gene flow and low migration rates found between *M. vison* populations in Spain, management strategies would be most effective still considering each mink population as a management unit. According to studies on introduced species (i.e., Miller et al. 2005) the invasive potential of a species increases when two different genetic lineages mix after secondary contacts. To avoid this scenario in Spain, efforts must be focused on the distribution borders and on the main potential corridors along river basins (such as the Ebro basin and other main rivers), trying to impede a connection among mink populations in the centre and north of Spain. Long-term monitoring programmes should also be conducted in a number of inhabited areas all over the mink range in Spain.

Acknowledgements This work is dedicated to Xavier Domingo-Roura, who passed away in November 2005; without his commitment and enthusiasm this study would have not been possible. We thank Ettore Randi for helpful suggestions on the molecular analyses. Most samples were obtained within European mink conservation projects

LIFE 00NAT/E/7299; LIFE 00NAT/E/7331; LIFE 00NAT/E/7335 and LIFE 02/NAT/E/8604 developed by the Governments of La Rioja, Junta de Castilla-Leon, Diputación Foral de Álava, Generalitat de Catalunya and Ministerio de Medio Ambiente. R. Lecis was supported by Ministerio de Educación y Ciencia, Madrid.

References

- Abdelkrim J, Pascal M, Calmet C, Samadi S (2005) Importance of assessing population genetic structure before eradication of invasive species: examples from insular Norway rat populations. *Conserv Biol* 19:1509–1518
- Anistoroaei R, Farid A, Benkel B, Cirera S, Christensen K (2006) Isolation and characterization of 79 microsatellite markers from the American mink (*Mustela vison*). *Anim Genet* 37:185–188
- Barbaresi S, Fani R, Gherardi F, Mengoni A, Souty-Grosset C (2003) Genetic variability in European populations of an invasive American crayfish: preliminary results. *Biol Invas* 5:269–274
- Belliveau AM, Farid A, O'Connell M, Wright JM (1999) Assessment of genetic variability in captive and wild American mink (*Mustela vison*) using microsatellite markers. *Can J Anim Sci* 79:7–16
- Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) GENETIX 4.02, logiciel sous Windows TM pour la genetique des populations. Lab Genome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Fr
- Benzecri JP (1973) L'analyse des donnees: T.2, L'analyse des correspondences. Dunoud, Paris, Fr
- Bonesi L, MacDonald DW (2004a) Impact of released Eurasian otters on a population of American mink: a test using an experimental approach. *Oikos* 106:9–18
- Bonesi L, MacDonald DW (2004b) Differential habitat use promotes sustainable coexistence between the specialist otter and the generalist mink. *Oikos* 106:509–519
- Bonesi L, Chanin P, MacDonald DW (2004) Competition between Eurasian otter *Lutra lutra* and American mink *Mustela vison* probed by niche shift. *Oikos* 106:19–26
- Bravo C, Bueno F (1992) Nuevos datos sobre la distribución del vison americano (*Mustela vison* Schreber) en España Central. *Ecología* 6:161–164
- Bryan MB, Zalinski D, Filcek KB, Libants S, Li W, Scribner T (2005) Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. *Mol Ecol* 14:3757–3773
- Brusgaard K, Malchenko SN, Christensen K, Lohi O, Kruse T (1998a) A polymorphic mink (*Mustela vison*) dinucleotide repeat. *Anim Genet* 29:467
- Brusgaard K, Holm LE, Lohi O (1998b) Two polymorphic mink (*Mustela vison*) dinucleotide repeat loci. *Anim Genet* 29:468
- Brusgaard K, Shukri N, Malchenko SN, Lohi O, Christensen K, Kruse T (1998c) Three polymorphic mink (*Mustela vison*) dinucleotide repeats. *Anim Genet* 29:153
- Clode D, MacDonald DW (2002) Invasive predators and the conservation of island birds: the case of the American mink *Mustela vison* and terns *Sterna* spp in the western Isles, Scotland. *Bird Study* 49:118–123
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144:2001–2014
- Coulon A, Guillot G, Cosson JF (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Mol Ecol* 15:1669–1679
- Craik C (1997) Long-term effects of North American mink *Mustela vison* on seabirds in western Scotland. *Bird Study* 44:303–309

- Davis CS, Strobeck C (1998) Isolation, variability and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. *Mol Ecol* 7:1771–1788
- Davison A, Griffiths HI, Brookes RC, Maran T, MacDonald DW, Sidorovich VE, Kitchener AC, Irizar I, Gonzalez-Esteban J, Ceña JC, Ceña A, Moya I, Palazon S (2000) Mitochondrial DNA and palaeontological evidence for the origins of the endangered European mink, *Mustela lutreola*. *Anim Conserv* 4:345–355
- Delibes M, Clavero M, Prenda J, del Carmen Blázquez M, Ferreras P (2004) Potential impact of an exotic mammal on rocky intertidal communities of northwestern Spain. *Biol Invas* 6(2):213–219
- Di Rienzo A, Peterson AC, Garza JC et al (1994) Mutational processes of simple-sequence repeat loci in human populations. *Proc Nat Acad Sci USA* 91:3166–3170
- Dunstone N (1993) *The mink*. T & AD Poyser Ltd., London
- Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Mol Ecol* 11(12):2571–2581
- Estoup A, Wilson IJ, Sullivan C, Cornuet JM, Moritz C (2001) Inferring population history from microsatellite and enzyme data in serially introduced cane toads, *Bufo marinus*. *Genetics* 159:1671–1687
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164:1567–1587
- Ferreras P, MacDonald DW (1999) The impact of American mink *Mustela vison* on water birds in the upper Thames. *J Appl Ecol* 36:701–708
- Fleming MA, Ostrander EA, Cook JA (1999) Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). *Mol Ecol* 8:1351–1362
- Fournier-Chambrillon C, Aasted B, Perrot A, Pontier D, Sauvage F, Artois M, Cassiede JM, Chauby X, DalMolin A, Simon C, Fournier P (2004) Antibodies to Aleutian mink disease Parvovirus in free-ranging European mink (*Mustela lutreola*) and other small carnivores from southwestern France. *J Wildlife Dis* 40(3):394–402
- Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to conservation genetics*. Cambridge University Press
- Garnier S, Alibert P, Audiot P, Prieur B, Rasplus JY (2004) Isolation by distance and sharp discontinuities in gene frequencies: implications for the phylogeography of an alpine insect species, *Carabus solieri*. *Mol Ecol* 13:1883–1897
- Gómez-Moliner BJ, Cabria MT, Rubines J, Garin I, Madeira MJ, Elejalde A, Aihartza J, Fournier P, Palazon S (2004) PCR-RFLP identification of mustelid species: European mink (*Mustela lutreola*), American mink (*M. vison*) and polecat (*M. putorius*) by analysis of excremental DNA. *J Zool Lond* 262:311–316
- Goudet J (1995) A program for estimating and testing gene diversities and differentiation statistics from codominant genetic markers. *J Heredity* 86(6):485–486
- Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Mol Ecol* 11:1103–1114
- Grapputo A, Boman S, Lindstrom L, Lyytinen A, Mappes J (2005) The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Mol Ecol* 14:4207
- Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. *Mol Ecol Notes* 5:708–711
- Hampton JO, Spencer PBS, Alpers DL, Twigg LE, Woolnough AP, Doust J, Higgs T, Pluske J (2004) Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *J Appl Ecol* 41(4):735–743
- Hansen MM, Jacobsen L (1999) Identification of mustelid species: otter (*Lutra lutra*), American mink (*Mustela vison*) and polecat (*Mustela putorius*), by analysis of DNA from faecal samples. *J Zool Lond* 247:177–181
- Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. *Genet Res* 38:209–216
- Holland BS (2000) Genetics of marine bioinvasions. *Hydrobiologia* 420(1):63–71
- Invasiber, Especies exóticas invasoras de la península Ibérica: <http://www.hidra.udg.es/invasiber/presentacion.php>
- Jonson RN, Starks PT (2004) A surprising level of genetic diversity in an invasive wasp: *Polistes dominulus* in the Northeastern United States. *Ann Entomol Soc Am* 97(4):732–737
- Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proc Nat Acad Sci USA* 75:2868–2872
- Lindholm AK, Breden F, Alexander HJ, Chan W, Thakurta SG, Brooks R (2005) Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. *Mol Ecol* 14(12):3671–3682
- Luikart GL, JM Cornuet (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv Biol* 12:228–237
- Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic changes. *Mol Ecol* 7:963–974
- MacDonald D, Strachan R (1999) *The mink and the water vole: analyses for conservation*. Wildcru and the Environment agency
- Mañas S, Ceña JC, Ruiz-Olmo J, Palazón S, Domingo M, Wolfenbarger JB, ME Bloom (2001) Aleutian mink disease parvovirus in wild riparian carnivores in Spain. *J Wildlife Dis* 37:138–144
- Maran T, Henttinen H (1995) Why is the European mink (*Mustela lutreola*) disappearing? A review of the process and hypotheses. *Ann Zool Fennici* 32:47–54
- Michaux JR, Libois R, Davison A, Chevret P, Rosoux R (2004) Is the western population of the European mink (*Mustela lutreola*) a distinct management unit for conservation? *Biol Conserv* 115:357–367
- Michaux JR, Hardy OJ, Justy F, Fournier P, Kranz A, Cabria M, Davison A, Rosoux R, Libois R (2005) Conservation genetics and population history of the threatened European mink *Mustela lutreola* with an emphasis on the west European population. *Mol Ecol* 14:2373–2388
- Miller N, Estoup A, Toepfer S, Bourguet D, Lapchin L, Derridj S, Reynaud P, Furlan F, Guillemaud T (2005) Multiple transatlantic introductions of the Western Corn Rootworm. *Science* 310:992
- Muñoz AR, Barbosa AM, Farfán MA, Real R (2005) Determinación de las áreas de expansión potencial del visón americano (*Mustela vison*) en España peninsular. VII Jornadas SECER, Valencia
- Nordstrom M, Hogmander J, Laine J, Nummelin J, Laanetu N, Korpimäki E (2003) Effects of feral mink removal on seabirds, waders and passerines on small island in the Baltic Sea. *Biol Conserv* 109(3):359–368
- O’Connell M, Wright JM, Farid A (1996) Development of PCR primers for nine polymorphic American mink *Mustela vison* microsatellite loci. *Mol Ecol* 5:311–312
- Palazon S, Ruiz-Olmo J (eds) (1997) *El visón europeo (Mustela lutreola) y el visón americano (Mustela vison) en España*. Ministerio de Medio Ambiente
- Palazon S, Ceña JC, Mañas S, Ceña A, Ruiz-Olmo J (2002) Current distribution and status of the European mink (*Mustela lutreola* L 1761) in Spain. *IUCN Species Survival Commission* 26:9–11
- Park K (2004) Assessment and management of invasive alien predators. *Ecol Soc* 9(2):12
- Peel D, Ovenden JR, Peel SL (2004) NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department Primary Industries and Fisheries
- Peebles JW, Fendley TT, Baker OE, Butfiloski JW (2002) Dispersal, home range and survival of repatriated mink in the northern

- coastal marshes of South Carolina. Proceedings of the 56th Annual Conference Southeastern Association Fish Wildlife Agencies, 219–228
- Peltier D, Lodé T (2003) Molecular survey of genetic diversity in the endangered European mink *Mustela lutreola*. C.R. Biologies 325:49–53
- Pinceel J, Jordanes K, Houtte N, Bernon G, Backeljau T (2005) Population genetics and identity of introduced terrestrial slug: *Arion subfuscus* s. l. in the North-east USA (Gastropoda, Pulmonata, Arionidae). Genetica 125:155–171
- Previtali A, Cassini M, MacDonald D (1998) Habitat use and diet of the American mink, *Mustela vison*, in Argentinian Patagonia. J Zool 246:482–486
- Pritchard JK, Stephens M, Donnelly PJ (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
- Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote excess in progeny. Genetics 144:383–387
- Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283
- Rousset F (1996) Equilibrium values of measure of population subdivision for stepwise mutation processes. Genetics 142:1357–1362
- Ruiz-Olmo J, Palazon S, Bueno F, Bravo C, Munilla I, Romero R (1997) Distribution, status and colonization of the American mink *Mustela vison* in Spain. J Wildlife Res 2:30–36
- Rushton SP, Barreto GW, Cormack RM, MacDonald DW, Fuller R (2000) Modelling the effects of mink and habitat fragmentation on the water vole. J Appl Ecol 37(3):475–490
- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu RevEcol Syst 32:305–332
- Schlaepfer MA, Sherman PW, Blossey B, Runge MC (2005) Introduced species as evolutionary traps. Ecol Lett 8:241–246
- Schneider S, Roessli D, Excoffier L (2000) Software for population genetics data analysis, including genetic differentiation and analyses of molecular variance (AMOVA). University of Geneva, Switzerland
- Sidorovich V, Kruuk H, MacDonald DW (1999) Body size and interactions between European and American mink (*Mustela lutreola* and *M. vison*) in Eastern Europe. J Zool Lond 248:521–527
- Sidorovich V, MacDonald DW (2001) Density dynamics and changes in habitat use by the European mink and other native mustelids in connection with the American mink expansion in Belarus. Netherland J Zool 51:107–126
- Stepien CA, Brown JE, Neilson ME, Tumeo MA (2005) Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for Risk analysis. Risk Anal 25(4):1043–1060
- Tyler C, Clark E, Pullin AS (2005) Do management interventions effectively reduce or eradicate populations of the American mink *Mustela vison*? Systematic Review N 7. Centre for evidence-based Conservation, Birmingham, UK
- Travis JMJ, Park KJ (2004) Spatial structure and the control of invasive alien species. Anim Conserv 7:321–330
- Vincent IR, Farid A, Otieno CJ (2003) Variability of thirteen microsatellite markers in American mink (*Mustela vison*). Can J Anim Sci 83:597–599
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370
- Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163(3):1177–1191
- Wisely SM, Buskirk SW, Fleming MA, McDonald DB, Ostrander EA (2002) Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. J Heredity 93(4):231–237
- Zeisset I, Beebe TJ (2003) Population genetics of a successful invader the marsh frog *Rana ridibunda* in Britain. Mol Ecol 12:639–646