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Mixture or mosaic? Genetic patterns in UK grey squirrels support a human-mediated 'long-jump' invasion mechanism

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ABSTRACT

Aim Clarifying whether multiple introductions of a species remain relatively isolated or merge and interbreed is essential for understanding the dynamics of invasion processes. Multiple introductions from different sources can result in a mixture of genetically distinct populations, increasing the total genetic diversity. This mixing can resolve the 'genetic paradox', whereby in spite of the relatively small numbers of introduced individuals, the augmented diversity due to this mixing increases adaptability and the ability of the species to spread in new environments. Here, we aim to assess whether the expansion of a successful invader, the Eastern grey squirrel, was partly driven by the merger of multiple introductions and the effects of such a merger on diversity.

Location UK, Ireland.

Methods We analysed the genetic variation at 12 microsatellite loci of 381 individuals sampled from one historical and 14 modern populations of grey squirrels.

Results Our data revealed that current UK population structure resembles a mosaic, with minimal interpopulation mixing and each element reflecting the genetic make-up of historic introductions. The genetic diversity of each examined population was lower than a US population or a historical UK population. Numbers of releases in a county did not correlate with county-level genetic diversity. Inbreeding coefficients remain high, and effective population sizes remain small.

Main conclusions Our results support the conclusion that rapid and large-scale expansion in this species in the UK was not driven by a genetic mixing of multiple introduced populations with a single expansion front, but was promoted by repeated translocations of small propagules. Our results have implications for the management of grey squirrels and other invasive species and also demonstrate how invaders can overcome the genetic paradox, if spread is facilitated by human-mediated dispersal.

Keywords
admixture, alien species, invasive, long-jump dispersal, *Sciurus carolinensis*, translocations.

INTRODUCTION

The release of a few individuals of an alien species in a new area leads sometimes to what some authors call a 'genetic paradox', that is the ability of bottlenecked populations with low genetic diversity, low evolutionary potential and low reproductive fitness to become invasive (Frankham, 2005; Roman & Darling, 2007). Genetic diversity in an introduced

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population is shaped by several interacting processes. Founder effects and stochastic extinctions will lead to a reduction of genetic diversity over time. Multiple introduction events, on the other hand, could increase diversity if populations mix at a later stage (Genton *et al.*, 2005; Lockwood *et al.*, 2005). Recent work has shown that the genetic paradox can be explained by the merging of propagules from genetically differentiated source populations that lead to increased diversity compared to the source population (Lockwood *et al.*, 2005; Simberloff, 2009), as is for example the case for anole lizards introduced in Florida (Kolbe *et al.*, 2004) and ladybirds introduced in the USA (Kajita *et al.*, 2012). Anole lizards showed higher local genetic diversity in the invaded area than lizards in their native range, following the merging and interbreeding of at least eight introductions from different source locations, thus explaining higher genetic diversity and migration rates, and outstanding invasion success of the species.

Biological and ethological characteristics such as reproductive strategies, ability to overcome ecological barriers or propensity for human-mediated dispersal may influence the admixing processes, and therefore the invasiveness, of introduced populations (Dlugosch & Parker, 2008). Genetic data combined with other information such as demography and historical records can be used to investigate the causes/mechanisms of spread of an invasive species (Suarez *et al.*, 2001; Wilson *et al.*, 2009).

The American Eastern Grey Squirrel (*Sciurus carolinensis*) (henceforth called the 'grey squirrel') in the British Isles provides a unique opportunity to examine how the invasion process can be influenced by multiple introductions of few individuals across large spatial scales, and what factors shaped the genetic diversity and structure of modern populations. In the British Isles, grey squirrels were once considered an ornamental species, and consequently, there have been multiple introductions from the native range, translocations from thriving introduced populations and natural spread. The history of grey squirrel introductions prior to 1930 was recorded by Middleton (1931), who listed a minimum of seven introductions from North America, ten translocation from previously established introduced populations in Great Britain and 12 introductions or translocations from unknown sources (Fig. 1 and Table S.1 in Supplementary Information). The most important introduction occurred in 1890, when 10 grey squirrels imported from New Jersey were released at Woburn Abbey, Bedfordshire. Woburn squirrels were later distributed as gifts across a minimum of seven sites in the UK and Ireland, but no systematic documentation of these translocations was kept, so the number of propagules from Woburn may have been greater. To complicate matters, individuals were sometimes translocated from these secondary introductions to new destinations. In the 1930s, Middleton (1931) identified three main expanding nuclei in the UK: the Midlands, Cheshire and Yorkshire. In the 1950s, Shorten (1954) recorded the expansion and merging of these three nuclei and reported three more expanding nuclei in Scotland. In Ireland, Boyd-Watt (1923a) documented the first stages of squirrel expansion from one translocation from Woburn (Table S.1). At present, grey squirrels have spread to occupy all of England (Usher *et al.*, 1992), most of Scotland with the exception of part of the Highlands (Bryce, 1997), and all Ireland east of the river Shannon (Carey *et al.*, 2007).

In this paper, we combine multilocus genotype data with demographic and historical records to track back the source and founder size of different propagules. Furthermore, we analyse patterns of genetic variation of descended and historical source populations and examine key demographic factors such as numbers of founders, effective population sizes and migration rates to explain the current genetic population structure. Our objective was to better understand the patterns and processes involved in a successful invasion and their consequences for genetic diversity and inbreeding. To do so, we apply population genetic approaches to assess possible pathways that led to the expansion of this successful invader in the UK. In particular, 1) we examined whether the grey squirrel invasion process was enhanced by the mixing of different propagules with different genetic origins, leading to the expansion of a homogenous population with high genetic diversity and 2) we also examined alternative invasion pathways. In particular, we assessed whether the multiple human-mediated translocations of propagules from the UK had a critical effect in enhancing the invasion process. Overall, the aim of this work was to provide insights into the mechanisms modelling standing genetic diversity and differentiation and the evolutionary potential of invasive species in general.

METHODS

Sampling and genotyping

Eleven individuals from 1921 to 1922 stored at the Natural History Museum (NHM), London, were used to represent the historical population from Woburn Abbey, three decades after it was introduced (Table S.2 in Supplementary Information), to assess changes in genetic diversity across time. DNA samples from modern populations were obtained from culling schemes in 2011. Modern populations were selected on the basis of presence of squirrel control schemes and proximity to at least one of the known original release sites from Woburn according to Middleton's (1931) report (Fig. 1, Table 1). These include, among others, populations from the park in Woburn Abbey itself (abbreviated as WA or OW, modern and historical populations, respectively), where a release in 1890 occurred; and Burnham Beeches (BB), Buckinghamshire, reported by Boyd-Watt (1923a,b) as a squirrel 'stronghold'. In addition, four more locations whose origin is unrecorded from Cambridgeshire (CA), Surrey (S), Northamptonshire (NA) and Cornwall (C) were included in the analysis (Table 1). A population from West Virginia (WV), USA (part of the grey squirrel's native range), was

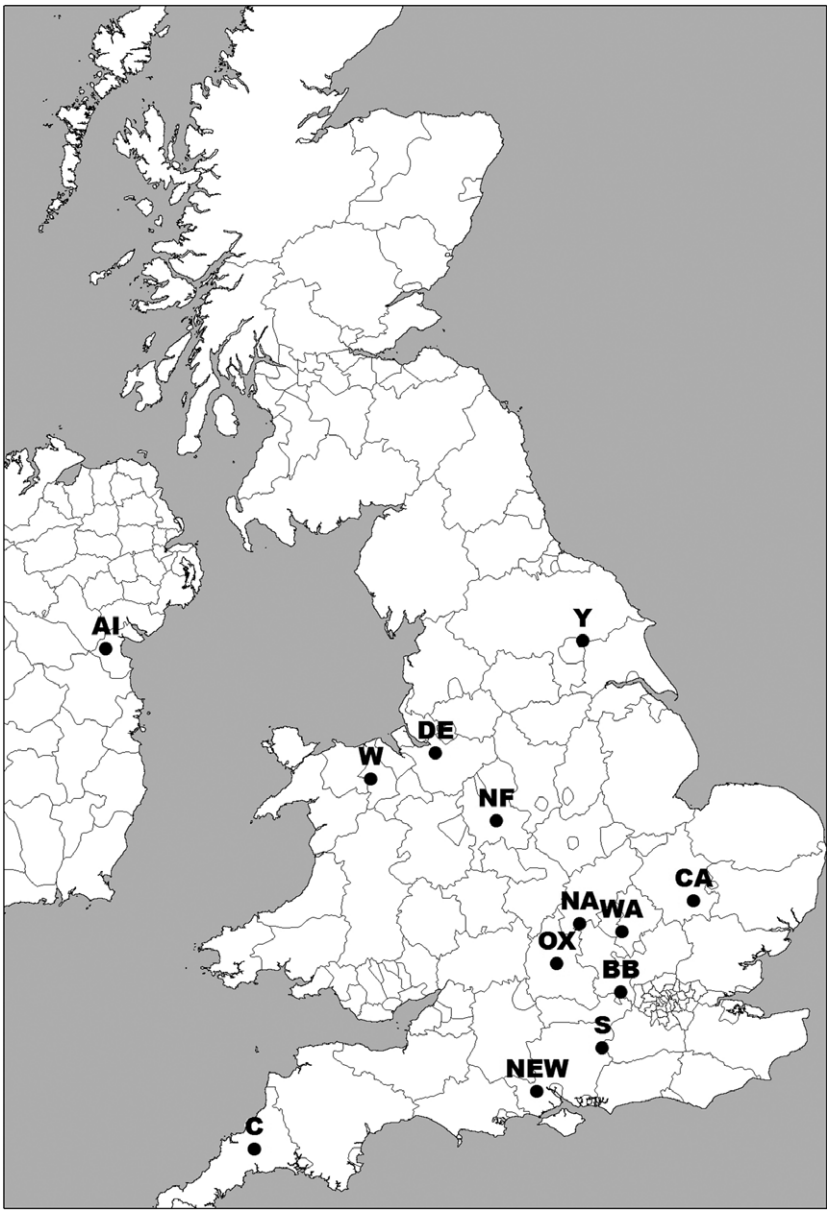

Figure 1 Grey squirrel populations (black dots) were selected for genetic analysis as close as possible to the introductions recorded by Middleton (1931).

used for comparison in some analyses. Richmond Park and Regent's Park, where the two largest introductions occurred, are now in the London urban area, where sampling was not allowed.

Tissue samples were collected from ear tips of individuals from modern populations. DNA was extracted from 5 to 6 mg of tissue using a Wizard SV 96 Genomic DNA Purification System (Promega) according to the manufacturers' protocols. Skin or brain tissue obtained from museum samples was <10 mg; DNA was extracted with QIAamp DNA Micro Kit (QIAGEN) using the manufacturer's tissue protocol with some modifications, as described in (Signorile *et al.*, 2014).

Twelve microsatellite markers were selected based on their capacity to amplify *S. carolinensis* DNA, their polymorphism and the absence of genotyping errors such as stuttering and allelic dropout (for details see Signorile *et al.*, 2014). The 12 primers were arranged in three multiplexes. PCR mixtures consisted of 4 μL of Multiplex PCR Kit (QIAGEN), 10–20 ng of template DNA and 1 μL of the chosen mix of primers (each primer concentration was 2 μM). Three different thermal cycle protocols were used for the three multiplexes, as described in (Signorile *et al.*, 2014). Diluted PCR products were run with an ABI Prism 3100 Genetic Analyser (Applied Biosystems). Alleles were scored with GeneMapper 3.5. To reduce genotyping errors, all genotypes were repeated at least twice and positive and negative controls were added to each PCR plate.

Statistical analysis

FSTAT for Windows (2.9.3.2) (Goudet, 1995) and GENEPOP 4.1 (Raymond & Rousset, 1995) were used to test for deviations from Hardy–Weinberg equilibrium and linkage

Table 1 Forests, counties, geographic position, number of individual grey squirrels sampled for this study and genetic parameters for each of the sampled populations.

Code	location	County	Lat	Long	N	H_E	H_O	P-val	F_{is}	F_{is} P-val	A_r	A_r SE	PA_r	F_{ST}
OW	Woburn Abbey 1922	Bedfordshire	51.983	−0.591	13	0.7674	0.6807	0.0012	0.119	0.0026	5.2	0.4099	0.39	0
WA	Woburn Abbey 2011	Bedfordshire	51.983	−0.591	30	0.7629	0.7411	0.1047	0.029	0.13	5.15	0.2898	0.18	0.0502*
BB	Burnham Beeches	Buckinghamshire	51.563	−0.629	25	0.7493	0.7233	0.0317	0.036	0.1193	4.82	0.3292	0.21	0.0572*
Y	Sand Hutton	Yorkshire	54.010	−0.937	30	0.7411	0.7087	0.0063	0.044	0.049	4.83	0.3118	0.2	0.0732*
CA†	Fulbourn	Cambridgeshire	52.179	0.226	30	0.7303	0.6675	0.0251	0.087	0.0023	4.61	0.4020	0.11	0.0838*
OX	Wytham Woods	Oxfordshire	51.775	−1.336	30	0.7303	0.6675	0.0017	0.087	0.0023	4.61	0.4020	0.11	0.0838*
S†	Alice Holt Station	Surrey	51.179	−0.852	30	0.7173	0.6403	0.0000*	0.109	0.0005	4.55	0.3491	0.01	0.0457*
C†	Cardinham Woods	Cornwall	50.484	−4.667	15	0.7092	0.6722	0.2138	0.054	0.0953	4.45	0.3293	0.01	0.0721*
DE	Delamere Forest	Cheshire	53.248	−2.680	30	0.7085	0.7373	0.9452	−0.041	0.9146	4.32	0.4043	0.1	0.1091*
NA†	Hazelborough For.	Northamptons	52.046	−1.065	30	0.6915	0.6150	0.0000*	0.112	0.0003*	4.44	0.3294	0.04	0.1139*
W	Clocaenog	Denbighshire	53.067	−3.424	30	0.6692	0.6749	0.4820	−0.009	0.6172	4.13	0.3564	0.08	0.1235*
NF	National Forest	Staffordshire	52.774	−1.983	7	0.6599	0.6012	0.1383	0.096	0.0565	4.37	0.3693	0.02	0.1148
NEW	New Forest	Hampshire	50.888	−1.586	56	0.6348	0.5698	0.0001*	0.104	0.001	3.98	0.4120	0.04	0.1042*
AI	Tallanstown	Louth, Ireland	53.920	−6.547	30	0.5849	0.5450	0.2521	0.071	0.0299	3.55	0.3683	0.08	0.186*
WV‡	Buckhannon	West Virginia, US	38.973	−80.348	23	0.7910	0.7174	0.0001*	0.095	0.0005	5.96	0.5278	0.56	0.0216

H_E, expected heterozygosity; H_o, observed heterozygosity; P-val, P values for the Hardy–Weinberg exact test for heterozygote deficiency, considered significant after Bonferroni correction with an adjusted nominal level of 0.0003 (for 5% significance, significant values are indicated with *); F_{is}, inbreeding coefficient; A_r, allelic richness; A_r SE, allelic richness standard error; PA_r, private allele richness; F_{ST}, fixation index, pairwise differentiation from OW. †Modern populations attributed to WA gene pool by assignment tests but not mentioned in historical sources; ‡Population from the native range.

equilibrium and to calculate *F*-statistics. All significance thresholds for nominal 5% significance were adjusted for type I errors from multiple testing using Bonferroni correction. Values of observed and expected heterozygosity were assessed with the Excel Microsatellite Toolkit (Park, 2001). Allelic richness (A_r) and allelic richness of private alleles (P_{Ar}) were estimated through rarefaction analysis with HP-RARE 1.1 (Kalinowski, 2005). The partial Bayesian approach implemented by ONCOR (Kalinowski *et al.*, 2007) was used for mixture analysis, to examine the relative composition of each population, using as a baseline only the populations examined for this study. Individual populations and the baseline were run with 1000 bootstraps with a 95% CI. STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) was also used to assign individuals to clusters using a Bayesian approach. A burn-in period of 25,000 Markov chain Monte Carlo steps was selected, followed by a chain of length 100,000. An admixture model with correlated allelic frequencies was chosen, with five independent iterations for each *K* value in the range 1–20 (Pritchard *et al.*, 2010). Analyses did not include the prior information of sampling locations. Following Pritchard *et al.* (2000), the posterior probability of the data for a given *K* value, Pr(X|*K*) (plotted as a function of *K* in Figure S.1), was used to determine the optimal *K* value.

The generation time and effective size (N_e) of squirrel populations were estimated by the software GONe 1.03 (Coombs *et al.*, 2012) using Jorde & Ryman (2007) unbiased estimator to assess the number of founders and the inbreeding levels. The software was designed to estimate these quantities for species with overlapping generations. We set three age classes in the software, Juveniles (<1 year old), Adults (1–2 years old) and Matures (2 years +). We then obtained survival and reproduction rate estimates from the literature (Shorten, 1954; Gurnell, 1987; Okubo *et al.*, 1989; Koprowski, 1994) of each age–sex class. We set the first age class as having a 25% survival rate and no reproduction for each sex. The second had an 80% survival of females and 70% survival of males, with all the surviving individuals reproducing. The third had a 50% survival of both males and females with all the surviving individuals reproducing. Jorde & Ryman (2007) estimator assumes that the temporal changes in allele frequencies were solely due to genetic drift, excluding any possibility of immigration. We therefore also estimated the effective population size and average migration rate using the temporal method implemented in the software MLNe (Wang & Whitlock, 2003). The most recent migration rates were also estimated by the Bayesian assignment method implemented in BayesAss 1.3 (Wilson & Rannala, 2003). The number of iterations used with BayesAss was 3,000,000, of which 999,999 were burn-in; the sampling frequency used was 2000.

The current inbreeding coefficient of each population derived from Woburn was calculated by

$$F = 1 - \Pi_{i=1}^{G}\left(1 - \frac{1}{2N(1+r)^{i-1}}\right) \text{(Signorile } et\ al.\text{, 2014)},$$

where *G* is the number of generations since the population was introduced from Woburn, in 1921–1922, *N* is the (effective) founder size, and *r* is the growth rate which is assumed constant. Generation time was estimated with GONe 1.03 as described above from the life traits of the species, and *r* was estimated from literature data as 0.82 (Okubo *et al.*, 1989). Given *F*, the proportional loss of heterozygosity since introduction is thus $1-F$. For populations with known founder sizes, the model predictions were checked against the F values calculated from the multilocus genotypes as $F = 1-H_t/H_O$, where H_t is the heterozygosity of modern populations and H_O is the heterozygosity of the Woburn 1922 population.

RESULTS

In total, 381 samples from 14 modern populations and one historic population were examined (Table 1; Fig. 1). Three British populations, Surrey, (S) New Forest (NEW) and Northampton (NA), and the population from the native range in the US showed heterozygosity deficiency after Bonferroni correction ($P < 0.0003$). Two loci (SCV6 and SCV31) were in linkage disequilibrium in populations from York (Y), Delamere Forest (DE) and Woburn Abbey 2011 (WA), ($P < 0.00005$).

The genetic diversity and the richness in private alleles of each population were examined. The population from the USA (WV) had the highest allelic richness (5.96) and private allelic richness (0.56) (Table 1). All the populations from the British Islands had a lower allelic richness than the historical and current Woburn populations (OW and WA, respectively). The historic population from 1922 and the current Woburn population (2011) have a similar allelic richness (5.2 ± 0.41 vs. 5.15 ± 0.29), but the former had a higher private allelic richness (0.39 vs. 0.18). The isolated Irish population has the lowest genetic diversity in terms of heterozygosity and allelic richness, showing a 31% allelic loss and a 23% loss in heterozygosity compared to the source population. The loss in allelic richness and heterozygosity of the Woburn 1922 population in comparison with the USA population, on the other hand, was far lower (12.7% and 3.8%, respectively). The pattern of private alleles (PA_r) (Table 1) among UK populations indicates that many of the alleles are actually shared even if on-going immigration is very low, supporting human-mediated translocations from the same source (Woburn). In addition, the high value for private alleles shown by the WV population also suggests that populations in the US are big and not homogeneous.

Mixture analysis was performed to examine the relative composition of each population (Table 2). These results show that the founder populations are still traceable in modern populations. The genetic contribution of WA is still detectable in the CA, S and AI populations. NEW was found largely to be derived from the nearby S population, despite a report by Middleton (1931) that the first squirrel introduc-

Table 2 Mixture analysis of the examined grey squirrel populations (columns), obtained with ONCOR (see methods for details). The rows show to which population each source on the left column contributed. Individual populations and the baseline were bootstrapped with a 95% C.I. Significant genetic contributions are shaded.

	Y	DE	W	NA	NF	WA	CA	BB	S	OX	NEW	C	AI
Y		0.0286	0	0	0.4576	0.0365	0	0	0.0336	0	0.109	0.0376	0.0361
DE	0		0.1382	0.003	0	0	0	0	0.0281	0	0	0	0
W	0	0.3093		0	0	0	0	0	0	0.1713	0.0325	0	0
NA	0.1014	0.1435	0.1255		0	0.0421	0.0939	0	0.0307	0.6159	0	0.1535	0.2591
NF	0.0821	0	0	0		0.0479	0.1902	0.1429	0	0	0.0766	0	0
OW	0	0	0	0	0	0.0625	0.0002	0.1439	0.1436	0	0	0.0004	0
WA	0.1971	0.2635	0.1633	0.143	0.1851		0.7155	0.1521	0.286	0.1353	0.0002	0.3013	0.6782
CA	0.0687	0	0	0.0387	0.2194	0.3118		0	0	0	0.1632	0	0
BB	0	0	0	0	0	0	0		0.0507	0	0.0406	0	0
S	0	0	0.0563	0	0	0.0978	0	0.1544		0	0.4032	0	0
OX	0.3927	0	0.44	0.7439	0.1377	0.1357	0	0.0834	0		0	0.3405	0.0266
NEW	0	0	0	0	0.0001	0	0	0	0.0911	0		0	0
C	0.0005	0.0224	0.0767	0.0715	0	0.0561	0	0.039	0	0.0774	0.1664		0
AI	0	0.0001	0	0	0	0	0	0	0	0	0	0	
WV	0.1574	0.2326	0	0	0	0.2096	0.0002	0.2843	0.3361	0	0.0084	0.1666	0

tion in Hampshire was from the London Zoo population, which was originally translocated from Woburn. S is the only population whose major genetic contributor is the American population, indicating an origin from an undetected introduction from the US, and poor subsequent mixing with other populations. WA significantly contributes (28.6%) to the S admixture, but is not relevant for the nearby BB population, halfway between the two locations and whose origin remains unclear. This indicates that grey squirrels were introduced from WA in S but not in BB. WA is also the main source for the population near Cambridge (CA) and, admixed with the very close NA nucleus, for the Irish population (AI), confirming that the Irish squirrels were imported from Woburn, as reported in historical sources (Boyd-Watt, 1923a). East Anglia was colonized by a recent eastwards expansion from the midlands, as described by Reynolds (1985). The presence of melanistic grey, or 'black' squirrels in Cambridgeshire (McRobie *et al.*, 2009), introduced in Woburn before 1930 (Shorten, 1954) is a phenotypic confirmation of the genetic evidence for the origin of this population. There are marked genetic exchanges between populations in Oxford (OX) and NA, but when NA is removed from the database the major contributor to OX is WA. The Welsh (W) and Yorkshire (Y) populations are admixed with the OX population, indicating undetected translocations from OX or, according to historical sources, translocations from Woburn, the same source as OX. NF, BB and C are not admixed with the examined populations, indicating origins from different introductions.

Despite their at least partially shared common ancestry with Woburn, all the examined modern populations are significantly differentiated from the historical Woburn population with the exception of the population from Surrey (S) and the population from the USA (WV) (Table S.3). Pairwise F_{ST} values of current populations with the historical Woburn population ranged from 0.029 (WV, USA) to 0.188 (AI, Ireland). Although the Irish population is significantly differentiated from its putative source population, a low richness of private alleles indicates that genetic drift and isolation can explain differentiation better than unrecorded introductions from the USA or other translocations from Great Britain.

Most importantly, the high number of releases did not result in an increase in the genetic diversity of the sampled populations in the UK. Correlation between mean allelic richness and number of known grey squirrel introductions and translocations per county was not significant ($P = 0.127$, $R^2 = 0.126$).

Structure analysis reveals that the examined populations can be grouped in 9 clusters (Figs 2 and S.1.), indicating an overall high rate of differentiation consistent with low migration rates, small founder sizes and poor interpopulation mixing (Table 3). Both the historical and current Woburn populations are an admixture of most of the alleles shared by the other populations, being the source of the other differentiated populations. The populations in Cheshire (DE) and Wales (W) cluster together; the populations in Oxfordshire (OX), Northamptonshire (NA) and, to a lesser extent, Cornwall (C) also cluster together. CA and the National Forest (NF) have a substantial proportion of membership in the same cluster.

Migration rates from Woburn, averaged over the period between introduction and 2011 and including translocations events, range between 0.028 and 0.097 and are not correlated to the distance from Woburn ($P = 0.9279$, Adjusted $R^2 = -0.09905$). Recent migration rates from Woburn are lower than the averages, ranging between 0.001 (SD $\pm$ 0.003) and 0.015 (SD $\pm$ 0.022) (Table 3). Overall, BayesAss results show little recent squirrel movements and scarce genetic exchange even between nearby locations (Table S.4). How-

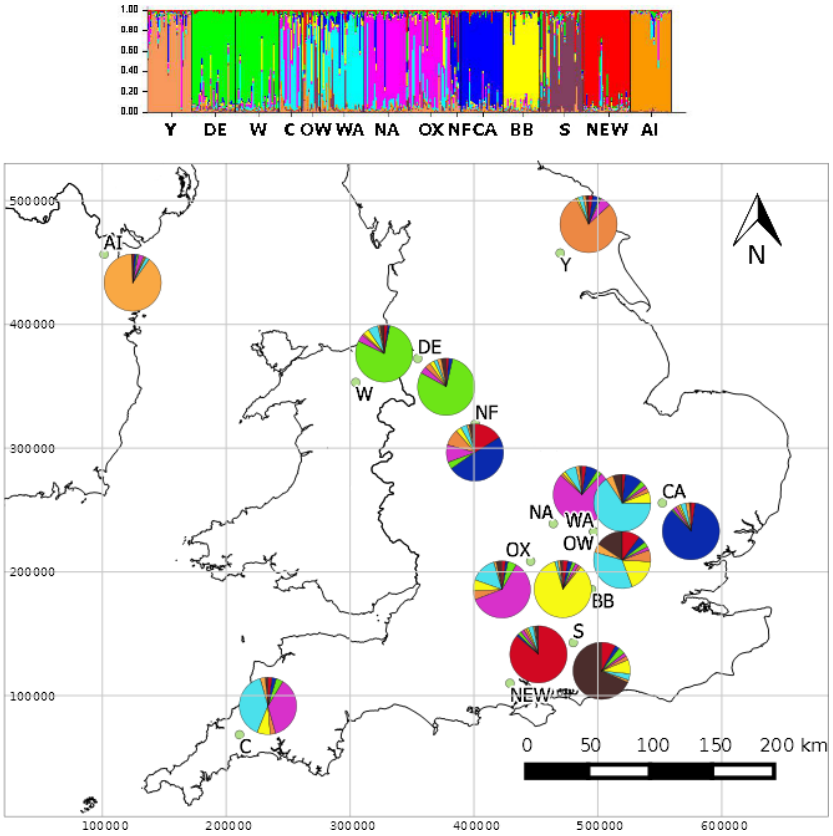

Figure 2 The average proportion of membership for the nine clusters identified by STRUCTURE, for each of the analysed populations. Each cluster is represented by a different colour. In the diagram, above each line represents an individual. See Fig. 1 and Table 1 for the locations and names of the sampling sites.

Table 3 Migration rates (m) estimated for the examined grey squirrel populations. Estimates from MLNe are averages in the period between the sampling in 1922 and the sampling in 2011 and include translocations. Estimates (SD in brackets) from BayesAss are the average migration rates in the previous two generations before the sampling in 2011. Migration rate m means the proportion of individuals a recipient population (the population following -> on the 1st column) receives from the source population (the population before -> on the1st column) in one generation.

	Distance (km)	MLNe	BayesAss	
WA->NA	33.3	0.0482	0.0015	(0.0045)
WA->BB	48.8	0.0475	0.0015	(0.0048)
WA->OX	56.5	0.0607	0.0035	(0.0073)
WA->CA	59.8	0.0785	0.0011	(0.0033)
WA->S	90.9	0.0319	0.0014	(0.0037)
WA->NF	130.6	0.0468	0.0149	(0.0219)
WA->NEW	140.7	0.0519	0.0011	(0.0030)
WA->DE	200.1	0.0604	0.0011	(0.0031)
WA->Y	229.2	0.0847	0.0023	(0.0055)
WA->W	229.3	0.0358	0.0011	(0.0033)
WA->C	331.1	0.0973	0.0080	(0.0144)
WA->AI	454.1	0.0283	0.0010	(0.0035)

ever, there are a few exceptions. The recent migration rate from NA to OX (0.278 ± 0.028) indicates an effective gene flow as a consequence of a natural squirrel dispersal and migration from OX appears to contribute to the genetic variation of the distant C and Y populations.

As all but one of the introductions mentioned by Middleton have a small founder size, the current grey squirrel populations must have experienced a bottleneck, substantial inbreeding and genetic drift. We measured this by estimating the population level inbreeding coefficient (equivalent to F_{st}, Methods). Using GONe 1.03 set as described in Methods, we estimated a generation time of 2.4 years, obtaining 37 squirrel generations between the original sampling in 1922 and 2011. We checked the model predictions against the F values of modern populations with known founder sizes, including four populations from this study and one population from the literature (Signorile *et al.*, 2014) (Table 4). Given that the predicted and calculated values fitted well in most cases, the model was used to estimate N, the number of founders, using the F calculated from the marker data. The predicted number of founders of the recent population in Woburn turns out to be much larger than the reported number of squirrels released in 1890, which is likely due to an unrecorded introduction. Populations NF, NEW and AI show a

Table 4 (Above) Comparison between the inbreeding coefficient (F) calculated from multilocus genotypes and from the model. Data on the number of founders come from the literature. Number of estimated founders obtained from calculated values of F for populations descending from introductions from Woburn and effective population size estimated with MLNe and GONe with the Jorde & Ryman (2007) unbiased estimator.

	Founders	F	F (model)
Y	36	0.0343	0.0305
AI	12	0.2378	0.0895
DE	10	0.0768	0.1067
NEW	6	0.1728	0.1732
Piedmont	4	0.2254	0.2514

Population	F	Founders (model)	Ne (MLNe)	Ne (GONe)
WA	0.0059	188	269.96	57.25
BB	0.0237	46	196.2	52.45
Y	0.0343	32	156.01	45.73
CA	0.0469	23	196.2	44.12
OX	0.0484	23	156.48	42.07
S	0.0654	17	251.81	58.27
DE	0.0769	14	135.62	36.35
NF	0.1402	8	125.77	41.4
NEW	0.1728	6	138.82	37.46
AI	0.2378	4	77.29	24.57

high level of inbreeding and a corresponding low number of founders. All of the examined populations show some level of inbreeding, with the exception of the modern Woburn population. The Irish population has a considerably lower effective population size compared to British counterparts.

DISCUSSION

The advancement of molecular and statistical methods offers an opportunity to detect past translocations when historic data are missing, and to understand the impacts of such translocations on the factors driving the spread and genetic structure of present populations (Fig. 3.). In contrast to the grey squirrels' reputation as a highly invasive species (Lowe *et al.*, 2000), our data indicate low migration rates, little gene flow, limited mixing of populations after introductions and, most importantly, multiple human-mediated translocations as a key driver for the species' success in the UK. The findings support the hypothesis that squirrel dispersal in the UK was mainly a consequence of human activities rather than innate grey squirrel propensity to spread and quickly invade new areas through the formation of a large, homogeneous expansion front. This also lends support to results obtained by Goheen *et al.* (2003), who carried out translocation and homing experiments on a number of squirrels species in the USA and suggested that dispersal behaviour of grey squirrels is sensitive to and constrained by habitat fragmentation and local patch size.

Our results show a reduction in allelic richness and heterozygosity in all the examined populations that were partially derived from past translocations from the same source in Woburn. The genetic diversity of a population founded from multiple introductions from different sources is expected to be similar or even higher than that of a single population from the native range (Kolbe *et al.*, 2004; Dlugosch & Parker, 2008), but our results clearly do not support this prediction for grey squirrels in the UK. Moncrief *et al.* (2012) showed that the phylogeographic history of grey squirrel evolution across their native range in the USA led to high haplotype diversity with no significant differentiation

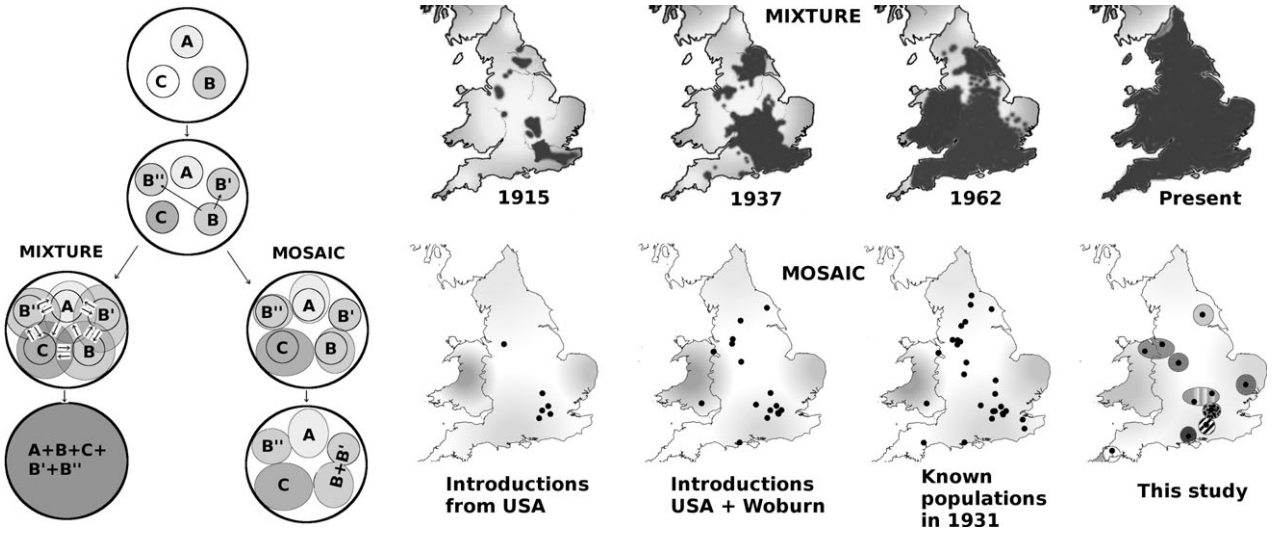

Figure 3 Schematic representing two alternative expansion models (Mixture and Mosaic) for introduced grey squirrels in the UK, assessed in this study. Left: diagram representing possible expansion models for species introduced with multiple propagules. Right: maps referring to the expansion mechanisms of the grey squirrel.

across the whole range. This lack of differentiation might have contributed to the limited genetic variation in the UK: multiple introductions from (likely) different sources might not have introduced new variation and different allelic patterns in the British populations. For western corn rootworm (Ciosi *et al.*, 2008) or for the velvet tree *Miconia calvescens* in the Pacific islands (Hardesty *et al.*, 2012), multiple introductions from the same source did not lead to an increase in genetic diversity. Our historical records do not specify the source of the introductions from North America, with the exception of the population in Woburn (New York or New Jersey) and one in West Scotland (Ontario, Canada). However, the low F_{st} value for WV compared to the OW population, and the high values for private alleles between the American and the UK populations suggest that there is some geographic structure in the US. Our data highlight that the initial introduction from the New Jersey source was from a very similar stock to the WV population, whereas the other introductions from the US to the UK might have come from genetically divergent US populations, which subsequently differentiated through drift. Further studies that test the differentiation of the American populations with microsatellites would be needed to understand if the low genetic diversity of the UK populations is due to the non-merging of the propagules, poor differentiation of the source populations in the native range or to both factors.

The observed population structure in the UK and Ireland appears mostly to be a consequence of genetic drift: it should be expected that the translocated propagules should, on average, constitute the same proportions of diversity as the source, except where stochastic processes such as drift have occurred. Our results indicate that significant levels of differentiation arise from the translocations from a single introduced population, confirming previous studies (Signorile *et al.*, 2014). Genetic drift increases the level of differentiation among locations by randomly changing allelic frequencies; its effects are especially strong for populations with severe bottlenecks (Kidd & Cavalli-Sforza, 1974). The relatively short time since the arrival of grey squirrels, low migration rates of the species, small effective population sizes suggest a major role for genetic drift over selection, adaptation, admixture and mutation in shaping patterns of genetic diversity of current grey squirrel populations in the UK and Ireland.

The genetic structure of grey squirrels currently is represented by a mosaic of different clusters that expanded to fill the gaps between translocations and shows limited interchanges of genetic material. It is not clear what limits the merging of these different clusters. A detailed study examining their boundaries would be needed to clarify the presence of ecological barriers or other factors such as regional control efforts that impact on squirrel densities. Grey squirrels in the UK are considered a forest pest species. For this reason, extensive and regular culling programmes have been carried out since the 1930s (Mayle *et al.*, 2007) which reduce population density and potentially dispersal. Ethological reasons also cannot be ruled out. Females may, for example, prefer dominant, resident males as mates rather than dispersing young individuals. As the grey squirrel becomes naturalized to the UK, however, it is likely that the different clusters will eventually merge, as happened in Pleistocene interglacial periods in the USA (Moncrief *et al.*, 2012). The necessary merging time will depend on the presence of suitable ecological corridors, which, in turn, will depend on anthropogenic activities and environmental factors.

Inbreeding is expected to occur during invasion processes, especially if propagule pressure is moderate (Allendorf & Lundquist, 2003). In this scenario, a possible inbreeding depression might have affected population growth and range expansion. Demographic stochasticity and the negative effects of inbreeding might have resulted in the extinction of many of the introduced or translocated populations mentioned by the historical sources, which were supposed to have had small founder sizes and consequently small effective population size. This may account for the absence of a positive relationship between number of founding events and genetic diversity in counties. An alternative hypothesis explaining how founder populations bounce back from bottlenecks, is purging selection (Facon *et al.*, 2011). The inbreeding process due to population bottlenecks associated with reintroduction could lead to inbreeding depression, but also to possible purging of rare recessive deleterious alleles which cause inbreeding depression (Frankham, 1995). If purging selection is effective enough, a population after a bottleneck could recover its fitness or even gain a higher fitness than its larger source population. However, effective purging can only occur with highly deleterious (e.g. lethal or semilethal) alleles, not with mildly deleterious alleles (Charlesworth & Charlesworth, 1987; Wang *et al.*, 1999). Unfortunately, a large proportion of inbreeding depression is believed to be caused by many marginally deleterious alleles. In Drosophila, for example, roughly half of the inbreeding depression in viability is due to many mildly deleterious mutations (Charlesworth & Charlesworth, 1987) for which purging selection is ineffective. The ineffectiveness of purging is partially confirmed by the observations of inbreeding depression in naturally selfing species (Agren & Schemske, 1993; Dole & Ritland, 1993) and by simulation studies (Hedrick, 1994; Wang *et al.*, 1999). We therefore assume purging is not a good explanation for the 'genetic paradox', at least in this case.

In is not clear why the modern WA population seems to have contributed to the modern populations more than the historical population. It is possible that the small number of available museum samples biased our results in this respect or that when the sampling occurred (1922) the Woburn population was differentiated and the sampled squirrels already showed some genetic drift. At least one other release occurred in Woburn prior to 1922 (Shorten, 1954), with animals carrying the gene for the black coat and this might have created some temporary fragmentation in the Woburn population.

Applications and outlook

Three main factors emerged from this study as determinant in controlling future grey squirrel expansions in countries such as Italy, where spreading and human-mediated translocations still occur: (1) human-mediated dispersal can play a major role in the spread and invasiveness of a species with low migration rates, (2) among populations with small founder size, genetic drift strongly affects allelic frequencies and inbreeding, leading to genetically structured populations when there is little or no interbreeding between subpopulations, and (3) the merging of different propagules does not appear to be an automatic process in biological invasions of squirrels.

The mechanisms of the expansion patterns of grey squirrels and other invasive species should be more thoroughly studied and included in predictive models and management policies. Although theoretical models have in many cases reasonably predicted invasion rates, violations of their assumptions may limit their usefulness (Suarez *et al.*, 2001). Many models predicting the expansion of grey squirrels (Okubo *et al.*, 1989; Rushton *et al.*, 1997; Lurz *et al.*, 2001) and other invasive species (Higgins & Richardson, 1996; Iordan *et al.*, 2012) frequently use a simple reaction–diffusion model with exponential growth. Individuals are assumed to disperse in each direction with equal probability and with the dispersal distance normally distributed within a fixed length of time (Kot *et al.*, 1996). Stochastic movements are seldom taken into account in predictive expansion range models (Lurz *et al.*, 2001; Bertolino *et al.*, 2008; Carrasco *et al.*, 2010).

The spreading mechanisms of invasive mammals and other terrestrial invasive vertebrates need to be further investigated. The human role in increasing the rate of spread of terrestrial vertebrates by stochastically introducing new propagules in areas distant from the original release sites is in most cases underestimated or ignored. Our results strongly supports the notion that humans were largely responsible for grey squirrel expansion through repeated introductions and translocations which appears to have been far more important than the initial introduction. Our study of the grey squirrel invasion of in the UK sheds new light on its role as an invasive species, with the surprising suggestions that it may not have the invasive potential one would predict by looking at the overall historic pattern of spread on its own. However, our results also indicate a potential threat, were subpopulations able to mix and increase their genetic diversity, leading to greater invasive potential (Signorile *et al.*, 2014).

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DATA ACCESSIBILITY

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S.1. Reported grey squirrel introductions and translocations between 1876 and 1931.

Table S.2. *Sciurus carolinensis* specimen samples from the NHM.

Table S.3. F_{ST} values of the examined grey squirrel populations.

Table S.4. Average migration rates from each analysed population.

Figure S.1. The posterior probability of the data for a given K value, $\Pr(X|K)$, as a function of K.

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