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BIODIVERSITY RESEARCH

Do founder size, genetic diversity and structure influence rates of expansion of North American grey squirrels in Europe?

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ABSTRACT

Aim This study investigates how founder size may affect local genetic diversity and spatial genetic structure of the invasive American eastern grey squirrel (*Sciurus carolinensis*) in European areas. It also examines whether dispersal propensity and invasion rate may be related to founder size, genetic diversity and structure.

Location Piedmont, Italy; Northern Ireland, Northumberland and East Anglia, UK.

Methods Across the invaded range in Europe, 315 squirrels from 14 locations, grouped in four areas, were sampled and examined at 12 highly polymorphic microsatellite loci. We estimated both genetic variation and population structure using AMOVA, Mantel tests and Bayesian analysis. We also estimated migration rates and range expansion rates.

Results Genetic diversity varied in accordance with numbers of founders across populations. For instance, the Italian population had the smallest founder size and lowest genetic variability, whereas Northumberland had high values for both. Significant levels of genetic differentiation were observed in all the examined regions. Gene flow, migration and population range expansion rate were also higher in England and Ireland than in Italy.

Main conclusions Populations descending from human-mediated releases of few individuals were more genetically depauperate and more differentiated than populations established from a greater number of founders. Propagule pressure is therefore a significant factor in squirrel invasions. There is a trend whereby larger founder sizes were associated with greater genetic diversity, more dispersal, less local genetic differentiation and faster range expansion rate in squirrels. These findings have important management implications for controlling spread rate of squirrels and other invasive species: good practice should prioritize preventing further releases and the merging of genetically distinct populations as these events can augment genetic diversity.

Keywords

Alien species, biological invasions, dispersal rate, genetic variation, invasive, microsatellites, propagule pressure, *Sciurus carolinensis*.

INTRODUCTION

A major question of invasion biology is why some invasions tend to be more successful and harmful than others. There is little consensus on the physiological and ecological characteristics of good invaders, and the relationship between intrinsic characteristics and invasion may be complex and of limited practical use (Kolar & Lodge, 2001; Crawford & Whitney, 2010). Instead, research indicates that one of the main factors influencing invasion success is the size of

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introduction events (Veltman *et al.*, 1996; Kolar & Lodge, 2001; Cassey *et al.*, 2004; Lockwood *et al.*, 2005; Sol *et al.*, 2007; Dlugosch & Parker, 2008a; Le Roux & Wieczorek, 2009; Hardesty *et al.*, 2012). Genetic bottlenecks at introduction can reduce genetic diversity, cause inbreeding depression and reduce adaptability to the new environment, limiting chances of successful invasion (Frankham *et al.*, 2002). When effective population size is low, stochastic loss of alleles is likely and drift can overcome selection. This can impact fitness and population dynamics (Lammi *et al.*, 1999; Reed & Frankham, 2003; Reed *et al.*, 2007; Roman & Darling, 2007). Conversely, many introduced individuals can lead to greater genetic variation, limiting inbreeding depression, promoting adaptation and facilitating invasion (Sakai *et al.*, 2001; Lee, 2002; Dlugosch & Parker, 2008a; Signorile *et al.*, 2014).

It is less clear, however, how founding events influence genetic diversity and genetic structure of invasive populations across their expanding range and how this, in turn, interferes with or promotes speed of colonization (Lee, 2002; Ramstad *et al.*, 2004; Myburgh *et al.*, 2007; Cameron *et al.*, 2008; Crawford & Whitney, 2010). Population genetic structure is a critical piece of information for effective management of invasive species because it provides a basis for defining management units, for assessing the levels of isolation among populations and for predicting future expansion. Knowledge of population genetic structure may also help identify populations that are more at risk of becoming invasive (Ramstad *et al.*, 2004), for example where invasion rate would be increased with additional introductions or mergers.

To better understand how population genetic mechanisms link founding events to expansion, it is important to understand why small founding events with low genetic diversity can lead to damaging invasions, as multiple-release events often do. The success of some very small introductions has been regarded as the effect of adaptive phenotypic plasticity, which overcomes the consequences of suboptimal conditions (Dlugosch & Parker, 2008b; Le Roux *et al.*, 2008), of wide environmental tolerance or of idiosyncratic-species-related advantages (Tsutsui *et al.*, 2000). This is, however, far from typical: most successful invasions derive from populations with a large propagule pressure and genetic diversity (Lammi *et al.*, 1999; Blackburn & Duncan, 2001; Reed & Frankham, 2003; Kolbe *et al.*, 2004; Lockwood *et al.*, 2005; Reed *et al.*, 2007; Roman & Darling, 2007; Simberloff, 2009). It is therefore important to examine the population genetic structure of invasions arising from different founder sizes and to relate genetic structure to propagule pressure to try to illuminate mechanisms that enable invasions.

The American eastern grey squirrel (*Sciurus carolinensis*) is an ideal model species to study biological invasions because it has been introduced with different numbers of individuals in different European countries and detailed records of introductions exist (Middleton, 1930, 1931; Martinoli *et al.*, 2010). It is one of the most invasive species world-wide (Lowe *et al.*, 2000); the knowledge gained here might also assist with the management of this invasive species.

Here, we examine population structure of the grey squirrel originating from widely differing release events in comparable landscapes. The populations we consider are in Piedmont, Italy, and in East Anglia, Northumberland and Northern Ireland, UK (Fig. 1). These populations originated from differing founder sizes from large, for example East Anglia and Northumberland, to very small, for example Italy (Table 1). In England, according to historical sources (Middleton, 1930, 1931), multiple introductions occurred in the 19th and 20th centuries. The last areas to be colonized by grey squirrels were Cumbria (Lowe, 1993, 2007; Lurz, 1995) and Northumberland, where populations arrived from the south in the late 1990s (National Biodiversity Network's (NBN) Gateway database). Grey squirrel spread has continued rapidly through these areas since then, and most forests in Northumberland have become colonized (Parrott *et al.*, 2009; Webber, 2011). The part of Northumberland we consider was colonized by an invasion front originating from Yorkshire (Lowe, 2007), to the south of Northumberland. At least four translocations occurred to Yorkshire in the early 20th century, establishing the population there (Middleton, 1931). Therefore, squirrel colonization in Northumberland originated from an admixed gene pool, and a large effective founder size can be assumed. In Ireland, only one introduction is known to have occurred, 12 individuals in 1911 at Castle Forbes, Co. Longford (Boyd-Watt, 1923). Grey squirrels have now colonized the eastern part of Ireland (Carey *et al.*, 2007). Colonization of Northern Ireland started in the late 1960s or the early 1970s from the south and has now reached the north coast (Tangney & Montgomery, 1995; Carey *et al.*, 2007). In Piedmont, Italy, four squirrels were released in Candiolo, near Turin, in 1948 (Martinoli *et al.*, 2010). After a 20-year lag phase with little expansion (Lurz *et al.*, 2001), squirrels began spreading but covered only 2016 km^2 by 2011 (S. Bertolino unpublished data). The spread of grey squirrels into East Anglia was recorded by Reynolds (1985) from 1960 to 1981. East Anglia was colonized from the south-west and possibly by a few releases in the easternmost part of the country. Only the south-west invasion is considered here.

An analysis of the effects of founder size on the genetic diversity and structure and colonization success of an introduced species has seldom been carried out because of the scarcity of records on founding events and the difficulty in obtaining genetic and spread rate data. The aim of this study is therefore to associate current genetic diversity, genetic structure and colonization success at local scales with the circumstances of past introductions obtained from detailed historical records. We focused on these specific questions: (1) How does squirrel genetic diversity at local scales vary across populations that sprang from different introduction events? (2) Given the examined range of founding events, the short time span since the introductions and the high vagility of the species, is it possible to observe substantial genetic differentiation among the sampling locations within the sampling areas, and how does the degree of differentiation appear to

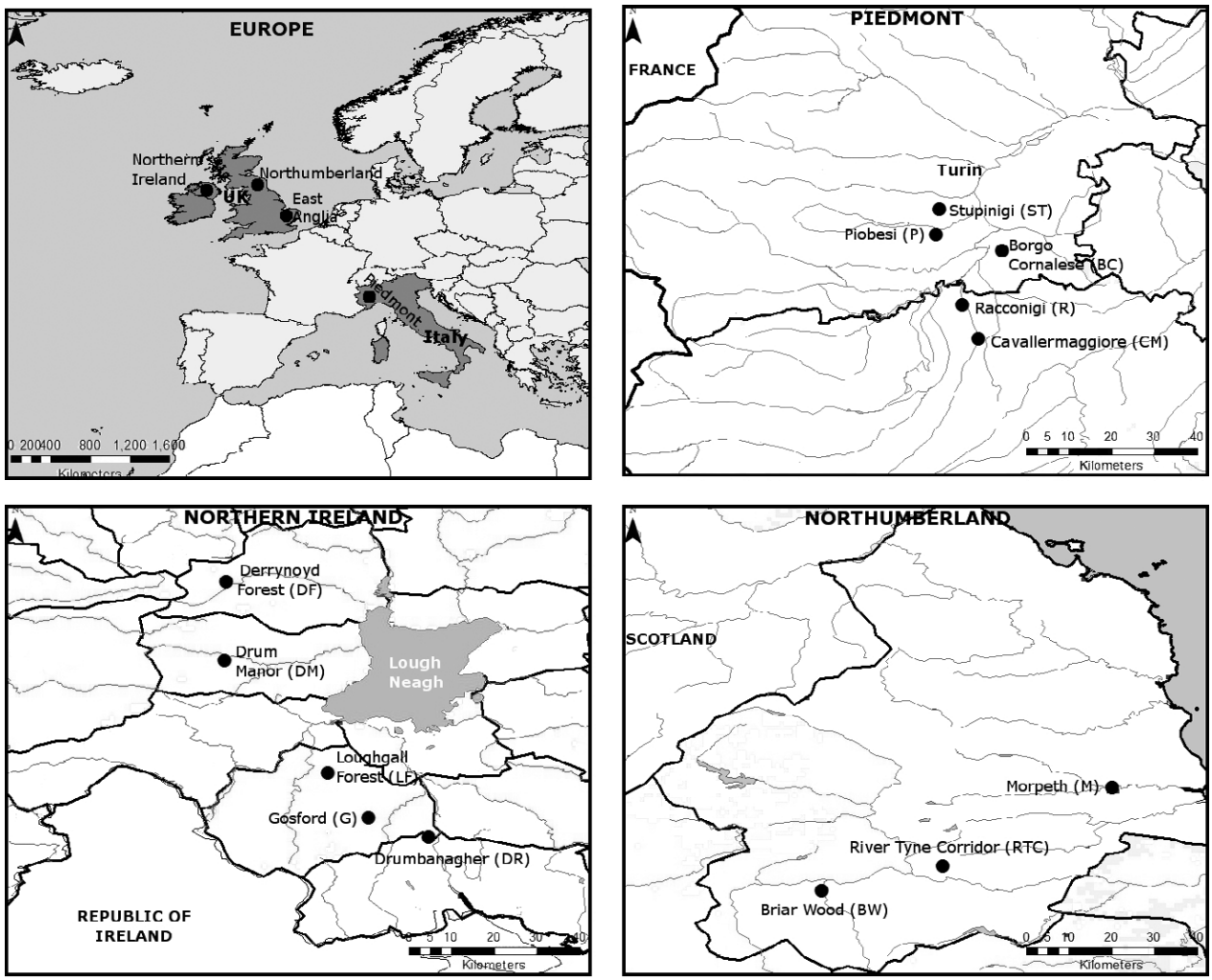

Figure 1 Maps showing the study areas, the additional population in East Anglia and the positions of the sampling locations in the areas.

relate to founder size and diversity? (3) Do the data suggest any relationship between founder size, genetic measures of dispersal propensity and population expansion rate?

METHODS

Study areas and sampling

The three main study areas and the additional population in East Anglia (Fig. 1; Table 1) were selected to span a range of founder sizes, as described in the Introduction. The term 'study area' refers to the sampled areas of Northumberland, East Anglia and Northern Ireland, UK; and Piedmont, Italy. All four study areas were plains crossed by rivers in fragmented habitats dominated by arable crops and meadows with interspersed broadleaf or mixed broadleaf and conifer woodlands. All sampling locations fell well within the range of altitude and climate conditions of the natural distribution of the species (Gurnell, 1987; Koprowski, 1994; Blackburn & Duncan, 2001; Gurnell *et al.*, 2004). The term 'location' or 'sampling location' refers to squirrels from the same woodland, within a study area (dots in Fig. 1 detail panels). Habitats in the study areas had similar fragmentation levels. Effective mesh size (M_{eff}) and mesh density (S_{eff}) were used by the European Environment Agency to measure fragmentation (EEA-FOEN, 2011), and we use their results as an approximate indicator of fragmentation as experienced by squirrels (Appendix S1 in supporting information for details).

DNA samples from Northern Ireland, Northumberland and East Anglia were collected from individuals culled for demographic control schemes in 2010–2011. The tip of one ear was stored in pure ethanol. In Piedmont, permits were obtained to capture and release the squirrels, and samples were 1-mm biopsy punches from the ear flap. Captures were carried out between 15 June and 15 August 2010, and in shorter sessions during 2011. Five additional samples were collected from road kills.

Table 1 Summary of the introduction histories of the examined squirrel populations

	Italy	Northern Ireland	Northumberland	East Anglia
Number of known introductions and translocations contributing	1, in Candiolo	1, in Castle Forbes, Co Longford	At least 31 recorded across the UK	At least 31 recorded across the UK
Number of animals recorded as being released during these introductions	4	12	> 353 total known across the UK	> 353 total known across the UK
Distance of sampling locations from point of introduction (in km)	3.7–27.6	104.6–134.4	NA	NA
Year squirrels were first recorded in the focal area	1948 in Candiolo	1911 in Castle Forbes	First seen in Northumberland in year 2001	First seen in East Anglia in year 1964

Introduction records are from Middleton (1931), Currado *et al.* (1987) and Martinoli *et al.* (2010). Data on arrival in East Anglia are from Reynolds (1985). Data on arrival in Northumberland are from the National Biodiversity Network's (NBN) Gateway database (http://data.nbn.org.uk). Introductions in Piedmont and Ireland were reported as single events. Multiple introductions occurred in England, and populations arrived in Northumberland and East Anglia via an expanding population range.

DNA samples from putative invasion sources or surrogates for sources were also gathered. We used as a surrogate source for the Piedmont squirrels a population from West Virginia; the Piedmont population was introduced from Washington, DC, close to West Virginia (Currado *et al.*, 1987). Twenty-three individuals from Buckhannon, West Virginia, were hunted as game and sampled. The population in Northern Ireland derives (Boyd-Watt, 1923; Middleton, 1931) from an introduction from Woburn, Bedfordshire, UK, in 1911. The populations in Yorkshire that spread into Northumberland sprang from at least four translocations, at least two of which were from Woburn Abbey (Middleton, 1930). The population in East Anglia is a recent spread from the squirrels in the Midlands, mostly from the modern population in Woburn Abbey. Therefore, 30 individuals from Woburn Abbey were culled as pests and sampled and used as a surrogate source population for East Anglia. Eleven Woburn Abbey skin fragments dating back to 1921–1922 and stored at the Natural History Museum in London were also sampled, representing the historical population from Woburn Abbey, and were used as a surrogate source for Northumberland and Ireland.

The 315 squirrels from 14 locations in the main study areas and 64 individuals from the putative sources were genotyped at 12 microsatellite loci, six originally developed for *Sciurus vulgaris* (Hale *et al.*, 2001) and six originally developed for *Sciurus Niger* (Fike and Rhodes 2009) (Lance *et al.*, 2003). Tests were run for Hardy–Weinberg equilibrium and linkage disequilibrium. Bonferroni corrections were made. Details are in Appendixes S2, S3 and Table S1.

Genetic diversity

Mean number of alleles (N_a), and observed (H_O) and expected (H_E) heterozygosity at each locus in each location were estimated with GENALEX 6.41 (Peakall & Smouse, 2006). Inbreeding coefficients (F_{IS}) were calculated and tested for significance with GENETIX 4.05 (Belkhir *et al.*, 2004). Allelic richness (A_r) and allelic richness of private alleles (P_{Ar}) were estimated with HP RARE 1.1 (Kalinowski, 2005), which uses rarefaction analysis to correct for different sample sizes.

Effective founder sizes

We estimated effective founder sizes of our populations as an attempt to corroborate the historically based estimates that the Piedmont population was based on four founders, the Irish population was based on 12, and the Northumberland and East Anglia populations were based on larger numbers of effective founders. Estimates of effective founder size, N, were obtained by numerically solving for N in the formula $F = 1 - \prod_{i=1}^{G} \left(1 - 1/2N(1+r)^{i-1}\right)$, where F is the current inbreeding coefficient (equivalent to the F_{ST} between the sampling area and the putative source), G is the number of generations since founding, and r is a growth rate. The formula is justified in Appendix S4. The generation time of a population with overlapping generations was estimated by the software GONE 1.03 (Coombs *et al.*, 2012), as described in Appendix S4. G was calculated as the number of years since introduction divided by generation time. The growth rate, r, was estimated from literature data as 0.82 (Okubo *et al.*, 1989). The current inbreeding coefficient, F, was estimated as $F = 1 - H_t/H_s$, where H_t and H_s are the heterozygosity of the sampling area and the source.

Genetic structure and gene flow

Pairwise genetic differentiation (F_{ST}) was calculated by GENEPOP 4.1 according to Weir & Cockerham (1984); FSTAT (Goudet, 1995) was used to test significance by bootstrapping over loci. Hierarchical analysis of molecular variance (AMOVA), as implemented in ARLEQUIN 3.11 (Excoffier *et al.*, 2005), was used for an initial assessment of genetic structures. The hierarchical levels used were within individuals, among individuals within locations, among locations within sampling areas and among sampling areas.

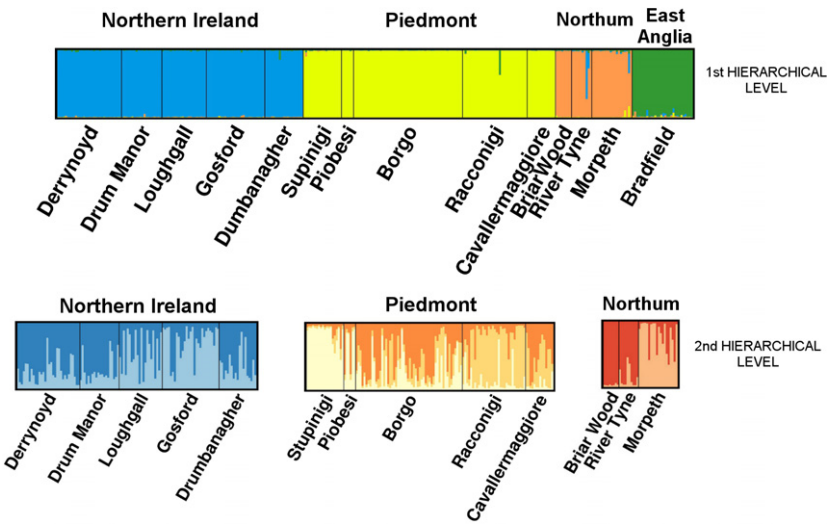

Figure 2 Structure analysis results based on 12 polymorphic microsatellites at two hierarchical levels. Each squirrel is represented by a vertical line whose colour represents its probability of membership in different clusters.

Significance of covariance components was assessed by 50,000 permutations.

The genetic structure of the grey squirrels was also examined at different hierarchical levels with STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), which uses genotypic data to assign individuals into a number of clusters, K. The most likely K is inferred as the value at which the greatest rate of change of the log probability of data between successive K values (ΔK) occurs (Evanno *et al.*, 2005). The analysis was carried out for the entire data set to determine the uppermost level of population structure. Runs were conducted with K between 1 and 14 to establish this uppermost structure. Not surprisingly, uppermost structure corresponded to sampling areas. To investigate nested structure, analyses of sampling areas were carried out (Fig. 2). For each analysis, we performed 20 independent runs for each K, to verify that estimates were consistent across runs, following Pritchard *et al.* (2010). For each run, the first 100,000 MCMC steps were discarded as burn-in, followed by 100,000 steps that were used. The admixture model and correlated allele frequency model were selected. Analyses did not include the prior information of sampling locations. STRUCTURE HARVESTER (Earl & VonHoldt, 2011) was used to examine STRUCTURE's output and to estimate the number of clusters.

Isolation by distance is defined as a decrease in genetic similarity between locations as the distance between them increases (Slatkin, 1993; Jensen *et al.*, 2005). This was assessed with a Mantel test within each area using the software 'Isolation by Distance Web Service' (Jensen *et al.*, 2005), by testing the correlations between Rousset's distance measure, $F_{ST}/(1 - F_{ST})$ (Rousset, 1997), and linear geographic distances. Significance was based on 10,000 permutations.

To assess dispersal in each study area, we used two programs for detecting first-generation migrants. As genetic differentiation between some of our sampling locations is low, the probability of 'misassignment' is relatively high. GENECLASS 2.0 (Piry *et al.*, 2004) was used with the method of Paetkau *et al.* (2004). For each squirrel, the ratio of the likelihood that it comes from the location from which it was sampled (home) to the highest likelihood value that it comes from any of the other sampled locations was checked. BAYESASS 1.3 (Wilson & Rannala, 2003) was also used to estimate migration rates. Software default parameters were used. As it was extremely unlikely to find first-generation migrants across sampling areas, methods were demonstrated not to be prone to type I errors by estimating the number of migrants among sampling areas, excluding East Anglia. As there were very unlikely to be true migration events, detections would have been type I errors. But, the methods made few such detections. Significance levels are 5% throughout this study.

Expansion rates

Assessing rates of spread of squirrels in different areas was complicated by patchy data from several sources gathered by different methods. However, spread rates as assayed visually by examining range maps appeared very different among our areas. Population range expansion was therefore assessed while taking into account uncertainties, to illustrate that differences between regions are generally larger than uncertainties. For each area, four pieces of information were assembled: first, a location where a population originated in an area, or a published range map; second, a 'start' date that was a year in which the population originated in the area or to which the range map applies; third and fourth, 'end' date and range were a population range estimate for a year after the start date. In the case of uncertainty in start dates, a range of possible dates were used. Approximate spread rate was calculated with a very similar method to Andow *et al.* (1990), by measuring 4–6 radii in different directions connecting the start state and end range, dividing by the time taken and averaging. Selected directions were approximately evenly spaced and were representative of the expansion. Maps and details are in Figs S1–S4. Uncertainties were incorporated through the uncertainties in start dates and by

Table 2 Locations and average measures of grey squirrel genetic diversity for each sampled location at 12 microsatellite loci

Locality	ID	Lat/Long	N	N_a	H_O	H_E	A_r	P_{Ar}	F_{IS}
Piedmont			125	3.83	0.53	0.55	2.92	1.03	0.04
Stupinigi	ST	54.80° 6.81°	19	3.33	0.57	0.52	2.89	0.12	−0.07
Piobesi	P	54.64° 6.82°	6	2.75	0.43	0.44	2.75	0.04	0.11
Borgo Cornalese	BC	54.40° 6.60°	54	3.25	0.57	0.54	2.84	0.01	−0.04
Racconigi	R	54.31° 6.51°	32	3.17	0.46	0.48	2.63	0.11	0.06
Cavallermaggiore	CM	54.27° 6.39°	14	2.83	0.48	0.46	2.63	0.03	0.01
Northern Ireland			122	5.42	0.54	0.57	3.52	1.20	0.05
Drumbanagher	DR	44.72° −7.68°	19	4.42	0.51	0.56	3.56	0.24	0.12
Gosford	G	44.79° −7.65°	29	4.25	0.51	0.51	3.13	0.04	0.01
Loughgall Forest	LF	44.91° −7.73°	22	4.42	0.56	0.57	3.56	0.16	0.05
Drum Manor	DM	44.94° −7.59°	20	4.17	0.57	0.56	3.49	0.09	0.02
Derrynoyd Forest	DF	44.99° −7.60°	32	4.17	0.54	0.52	3.14	0.08	−0.03
Northumberland			38	6.25	0.64	0.66	4.30	1.68	0.04
Morpeth	M	55.17° −1.70°	20	5.42	0.62	0.65	4.11	1.06	0.06
River Tyne Corridor	RTC	54.99° −2.11°	10	4.67	0.63	0.61	3.99	0.47	0.02
Briar Wood	BW	54.94° −2.32°	8	3.58	0.72	0.59	3.44	0.36	−0.15
East Anglia	EA	52.19° −0.83°	30	6.17	0.77	0.71	4.54	0.87	−0.07
Old Woburn	OW	51.98° −0.59°	11		0.68	0.77			
Modern Woburn	WA	51.98° −0.59°	30		0.74	0.76			
West Virginia	WV	38.97° −80.35°	23		0.72	0.79			

N, sample size; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; A_r, allelic richness; P_{Ar}, private allelic richness; F_{IS}, inbreeding coefficient. Locations are in order from closest to furthest from the initial point of introduction or along the route of the invasion front.

retaining individual spread rate measurements (Figs S1–S4 captions for details); ranges and standard deviations of spread rate estimates are presented.

RESULTS

Genetic diversity

Across the four sampling areas, the 12 loci had 111 alleles, each locus having 3 to 15 alleles, mean 9.25 alleles per locus. Locus SCV31 showed significant departure from Hardy–Weinberg equilibrium after Bonferroni correction (heterozygote deficit; $P = 0.0001$) in Drumbanagher, Northern Ireland (DR). This could be because of a sampling error due to small sample size or hidden genetic structure. Analysis with MICRO-CHECKER did not reveal null alleles for SCV31 so the locus was not excluded. No loci were found in linkage disequilibrium at $P < 0.0007$ (the probability level required after Bonferroni correction). Across all loci, no F_{IS} values were significantly different from zero (Table 2).

Measures of diversity, in general, were highest in the single population examined in East Anglia. In the three other areas, diversity was highest in Northumberland and lowest in Piedmont. All 12 loci were polymorphic, but SCV13 was monomorphic within Piedmont. Observed heterozygosity across all loci ranged from 0.43 (Piobesi, P, Piedmont) to 0.72 (Briar Wood, BW, Northumberland) and 0.77 (East Anglia). Average allelic richness (A_r) per sampling location ranged from 2.63 in Cavallermaggiore (CM) and Racconigi (R), Piedmont, to 4.11 in Morpeth (M), Northumberland, and 4.54 in East Anglia and was minimal in Piedmont (2.92) and maximal in Northumberland (4.3) and East Anglia (4.54) (Table 2, Fig. 3).

Measures of genetic diversity varied within, as well as among, sampling areas. In Piedmont, the highest A_r was in the original release point (Stupinigi, ST), and A_r was also high in Borgo Cornalese (BC). Genetic variation was not significantly correlated with distance from the release point ($P = 0.1116$, $R^2 = 0.6245$). In Northern Ireland, the lowest A_r values were in Derrynoid forest (DF), the northernmost of the locations and the furthest from the original release point, and in Gosford forest (G), a location that structure analysis indicated was isolated (Fig. 2; Table S2). Apart from Gosford, which is an outlier, the Irish locations showed that allelic richness significantly decreased with distance from release point ($P = 0.0306$, $R^2 = 0.9397$). In Northumberland, there was a gradient of increasing allelic richness from West to East (Briar Wood to Morpeth, $P = 0.0363$, $R^2 = 0.9968$); population range expansion occurred approximately from East to West (Fig. S3). The difference between Briar Wood and Morpeth was the highest observed within a sampling area. Morpeth had the largest private allelic richness (1.06; four private alleles).

Number of founders

We estimated three founders for Piedmont, four for Northern Ireland, eight effective founders for Northumberland and 45–51 for East Anglia, values that were consistent with the general ordering of founder sizes determined from literature.

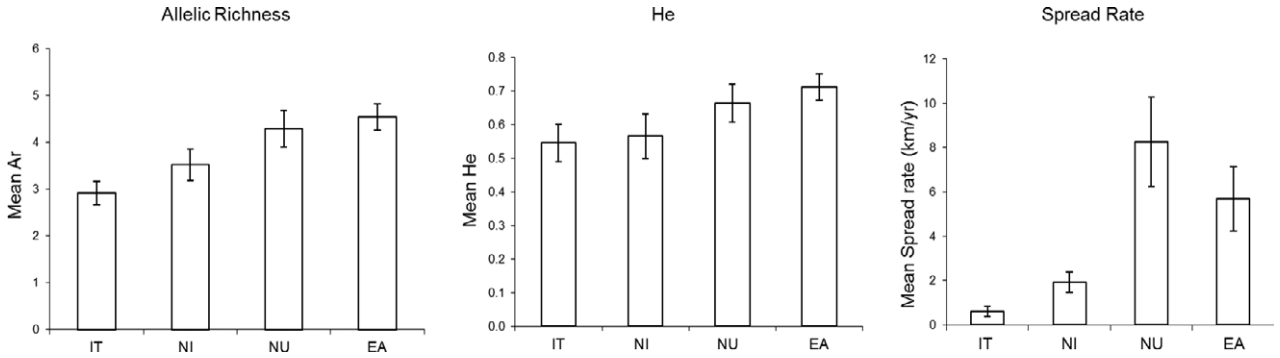

Figure 3 Genetic diversity indicators estimated at 12 polymorphic loci and average spread rate (km/yr) compared across study areas. IT, Piedmont; NI, Northern Ireland; NU, Northumberland; EA, East Anglia. Error bars are standard errors except for spread rates, for which standard deviations are given instead because non-independence of spread rate estimates makes standard errors inappropriate.

GONe estimated a generation time of 2.4 years. Because of uncertainties in the arrival time of squirrels into each region, we used two estimates and computed two possible founder sizes for each region (Table S3). These differed only for East Anglia.

Genetic differentiation

Among study areas, the level of genetic differentiation, measured by pairwise F_{ST} values, was always significant after Bonferroni correction and ranged from 0.118 (East Anglia and Northumberland) to 0.264 (Northern Ireland and Piedmont) (Table 3).

Within study areas, genetic structure was most pronounced in Piedmont and was less pronounced in Northumberland and Northern Ireland. In Piedmont, only the pairwise F_{ST} value between Stupinigi (ST) and Piobesi (P) was not significant (Table 3). Other pairwise F_{ST} values ranged between 0.038 and 0.149. This shows a high level of differentiation among sampling locations despite small founding size, close proximity of locations and the recent introduction of squirrels to the region and indicates restriction in gene flow. In Northern Ireland, two comparisons were non-significant and other F_{ST} values ranged between 0.013 and 0.061. Likewise, the level of differentiation was low in Northumberland (Table 3), indicating that in Northern Ireland and Northumberland some gene flow occurs between locations.

Hierarchical analysis of molecular variance showed that differences between study areas contributed 23% ($P < 0.01$) of the variation (Table 4). Variance among locations within areas was low but still significant (4%, $P < 0.01$). The East Anglia population was excluded from this analysis.

STRUCTURE partitioned the pooled samples from the four study areas into four clusters (K = 4) that correspond to the areas. Within each of the three main areas, STRUCTURE indicated three clusters in Piedmont, two in Northern Ireland and two in Northumberland (Fig. 2; Table S2). In Piedmont, although the $K = 3$ model was not supported by the highest ΔK value (Evanno *et al.*, 2005), it had the absolute maximal posterior probability (mean LnP(K) across the 20 repeats), suggesting three genetically distinct groups. Adding sampling location information to the structure analysis, the $K = 3$ model was the most supported.

Table 3 Pairwise Wrights F_{ST} values (below the diagonal) calculated from microsatellite data and geographic distances (above the diagonal, km) between study areas (top) and locations (bottom) for grey squirrel populations in Europe

	Piedmont	N. Ireland	Northumberland
N. Ireland	0.264*		
Northumberland	0.226*	0.204*	
East Anglia	0.170*	0.152*	0.118*

Piedmont	ST	P	BC	R	CM
ST	0	6.2	14	22.8	31.1
P	0.0257	0	11.6	16.9	25.5
BC	0.0719*	0.0408*	0	14.5	21.3
R	0.1187*	0.1045*	0.0575*	0	8.4
CM	0.1488*	0.1441*	0.0378*	0.0514*	0

N. Ireland	DF	DM	LF	G	DR
DF	0	18.4	47.3	59.9	65.3
DM	0.0316*	0	31.1	42.8	48.5
LF	0.0538*	0.0134	0	11.8	17.9
G	0.0612*	0.0450*	0.0161*	0	6.4
DR	0.0421*	0.0267*	0.0153	0.0327*	0

Northumberland	BW	RTC	M
BW	0	14.8	47
RTC	0.0159	0	32
M	0.0719*	0.0479*	0

*Bonferroni-corrected statistical significance at the 5% level.

Dispersal and migration rates

Mantel tests showed a strong and significant positive association of pairwise F_{ST} with distance ($r = 0.839$, $P = 0.017$) for

Table 4 Results of the analysis of hierarchical molecular variance (AMOVA) for squirrels across the invaded range in Europe. The East Anglia population was excluded from this analysis

Source of variation	d.f.	Sum of Squares	Variance components	% of variation	Fixation Index	*P*-values
Among sampling areas	2	385.058	1.043	23	Frt = 0.2280	< 0.01
Among locations within areas	10	110.732	0.183	4	Fsr = 0.0517	< 0.01
Among individuals within locations	272	940.694	0.110	2	Fis = 0.0328	< 0.05
Within individuals	285	923.000	3.239	71	Fit = 0.2920	< 0.01
Total	569	2359.484	4.574			

Piedmont, and a moderate and significant positive association ($r = 0.669$, $P = 0.031$) for Northern Ireland (Fig. 4). The test was not carried out for Northumberland or East Anglia because there were too few locations.

All individuals were correctly assigned back to their sampling area by BAYESASS 1.3 and all but one were correctly assigned by GENECLASS 2.0, indicating that the type I error rate is low when populations are substantially differentiated. Migration among locations within a study area was generally found to be lower in Piedmont than in the other two main study areas (Table S4). In Piedmont, percentage of first-generation migrants (Nm F_0, Table 5) ranged between 8.8% and 11.5%. On average, Northern Ireland migration rate was estimated as 14.5% by GENECLASS 2.0 and 26.6% by BAYESASS 1.3. A higher rate of gene flow is also supported by the low F_{ST} values measured across Northern Ireland. In Northumberland, the two assessment methods calculated Nm F_0 values 18.4% and 13.9%. However, the power to identify F_0 immigrants is low when populations are little differentiated and sample sizes are small; these estimates should be treated with caution. In any case, migration rates for Ireland and Northumberland were higher than for Piedmont.

Expansion rates

Our findings indicate a relationship between founding events and expansion. In Piedmont, population spread rate is substantially slower (mean value of our estimates 0.60 km/yr, maximum 1.05 km/yr) than for the English populations (Northumberland, mean estimate 8.25, minimum 5.78 km/yr; East Anglia, mean 5.70, minimum 3.57 km/yr), with an intermediate value for Ireland (mean 1.92, minimum 1.32, maximum 2.61). Differences between regions eclipse uncertainties: the maximum for Piedmont was less than the minimum for Northern Ireland, and the maximum for Northern Ireland was less than the minima for Northumberland and East Anglia. Figure 3 compares rates to genetic diversity: spread rate was generally higher for the locations with greater diversity.

DISCUSSION

Our analysis of the influence of propagule size on the genetic diversity and differentiation, gene flow and migration rates of grey squirrels in Europe contributes to our understanding

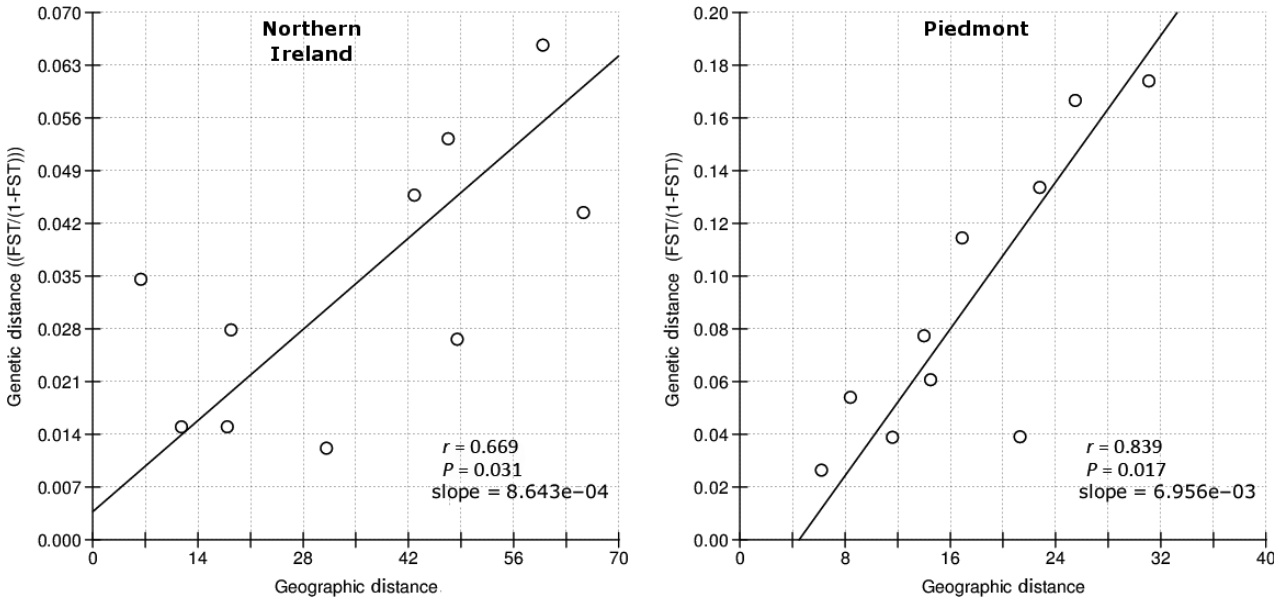

Figure 4 Isolation-by-distance assessment: scatter plot and linear interpolation of grey squirrel genetic distance versus Euclidean distance in Northern Ireland and Piedmont estimated through a Mantel test.

Table 5 Estimated absolute and relative number of grey squirrel migrants per generation in three European invaded regions obtained with two methods, and habitat fragmentation in each area (non-mountainous land areas only). See Appendix S1 for the definition of S_{eff}, a measure of fragmentation

	GENECLASS		BAYESASS		Habitat fragmentation 2009
	Nm		Nm		
	F_0	%	F_0	%	S_{eff}
Piedmont	11	8.8	14.4	11.5	10.17
N. Ireland	18	14.5	32.5	26.6	9.30
Northumberland	7	18.4	5.3	13.9	2.46

of the mechanisms by which founding events affect colonization success (Dlugosch & Parker, 2008a; Crawford & Whitney, 2010). Results show that genetic variation in four study areas varies in accordance with founder size, answering question 1 from the Introduction. This pattern is consistent even when founder size was as low as 4 or 12, and genetic diversity might have been levelled out by drift, bottlenecks or natural selection. Multiple analyses indicate that significant levels of differentiation arise from the spread of single introduction events, answering question 2 from the Introduction. This indicates reduced squirrel movement, with reductions greatest in areas with smallest founder size, answering question 3 in the affirmative. Our data indicate some of the steps by which founder size differences may translate into differences in expansion rate in the field. Although some results are limited by the small number of sampling areas, patterns are consistent across genetic diversity and differentiation, dispersal of individuals and range expansion rate and hence provide a basis to understand the impacts of genetics and founding events on population range expansion and can help guide future work.

The observed positive effect of propagule pressure on the genetic variance of introduced species is not universal (Poulin *et al.*, 2005; Myburgh *et al.*, 2007; Le Roux *et al.*, 2008). In some cases, multiple releases can give rise to low genetic diversity if all introductions came from the same source population or there was post-introduction selection (Tsutsui *et al.*, 2000; Grapputo *et al.*, 2005; Hardesty *et al.*, 2012). In the present data, however, grey squirrels have proven to be highly sensitive to the size of introduction events. Furthermore, the adaptive potential of genetically poor populations can be low, and this makes squirrels more vulnerable and appears likely to help explain slower expansion rates of populations with lower diversity. For instance, it may have contributed to the 20-year lag phase of little expansion in Piedmont. Grey squirrels in Italy and Ireland may be more vulnerable than in the UK to persistent control measures due to low adaptive potential caused by poor genetic diversity. Grey squirrels have proven to be very successful invaders generally, able to start new populations world-wide even from few founders (Wood *et al.*, 2007; Bertolino, 2009). This may be explainable through exaptation or phenotypic plasticity, as well as through inbreeding avoidance (Dlugosch & Parker, 2008a). But, our findings nevertheless suggest that genetic diversity, conditioned by founding events, still plays a role in spite of these other species advantages: genetic diversity can influence squirrel spread rates.

Alternative hypotheses exist that may explain observed patterns, but these seem less likely than the hypothesis that low founder sizes cause low genetic diversity, which in turn reduces individual migration and population spread rate. Firstly, a type I error is always possible, that is, that some unmeasured factor truly causes the differences in spread rate among our sampling areas, and this factor happens by chance to be aligned with founder size and the genetic measures we have considered. This is less likely because our results are consistent across more than one measure (genetic estimates of dispersal as well as direct measurements of range expansion). In our view, the main potential candidate for such a variable is habitat fragmentation, but we showed that our sampling areas have similar levels of fragmentation (Table 5; Appendix S1). Also, our results are consistent with a large body of work showing that propagule pressure is related to establishment and invasion success (Veltman *et al.*, 1996; Kolar & Lodge, 2001; Cassey *et al.*, 2004; Lockwood *et al.*, 2005; Sol *et al.*, 2007; Dlugosch & Parker, 2008a; Le Roux & Wieczorek, 2009; Hardesty *et al.*, 2012). Secondly, differences in genetic diversity could be a consequence of expansion rate differences, in addition to or instead of being their cause: faster-expanding populations could have had shorter bottleneck times, preserving more genetic variation. This is predicted by classic population genetics theory, assuming that range expansion rate is positively correlated with the rate of increase in effective population size (i.e. a larger range means a higher effective population size). This mechanism may have played some role in generating the genetic diversity patterns we observed. However, the good alignment between historical information on population founder sizes and measures of diversity and population expansion rate (see Appendix S5 for discussion of this agreement) suggests that expansion rate must have played a minor role at best in causing the observed genetic patterns.

Differentiation levels, gene flow results and observed isolation-by-distance patterns suggest that squirrels mostly move according to a stepping-stone model (Kimura & Weiss, 1964), that is, individuals go from habitat patches only to nearby patches, with individual migrants only seldom travelling large distances. Under a mainland-island or source-sink model (Pulliam, 1988), migrants would go from a source population to one or more sink populations roughly independent of the geographic distances. In this case, there should be no isolation by distance, heterozygosity levels should be lower in the sinks, and migration flow should be unidirectional; however, this scenario does not seem likely to describe our squirrel systems because we observed similar diversity and migration rates in all locations within a sampling area, and we detected isolation-by-distance patterns. As

population extinctions and successive recolonizations have not been described yet in our study areas, a metapopulation model (Hanski & Gilpin, 1991) would not fit our case study, either.

Global climate warming is causing widespread latitudinal and altitudinal species range shifts (Parmesan & Yohe, 2003), and these shifts can be rapid (Parmesan, 2006; Garroway *et al.*, 2011). Our results suggest the possibility that species or populations of low genetic diversity might have accentuated difficulty in shifting their range to keep up with climate shifts. Poleward and upward expansions under climate shifts are 'natural' in the sense that they do not follow human-mediated introductions of a species to a new area, as do the squirrel expansions we considered. But they are otherwise similar enough to the invasions/expansions studied here that we can reasonably speculate based on our results that poleward and upward expansion rates may be correlated with available genetic diversity for some species. Many species of conservation concern have low genetic diversity, suggesting that the source of climate-related risk identified here will be borne most heavily by those species least able to cope with it. Difficulties in carrying out rapid population range shifts due to low species genetic diversity would be in addition to any difficulties due to low natural dispersal ability or habitat fragmentation.

Our results have management implications, particularly for grey squirrels in Italy. The invasion in Italy today may be comparable, as a threat to native species, to the invasion in England of the early 1900s: distinct population nuclei are spreading but have not yet merged (Martinoli *et al.*, 2010). Although the Piedmont population is currently expanding slowly, it seems likely that expansion rates will accelerate once the population meets nearby nuclei in Lombardy and Liguria and diversity increases. This possibility is particularly worrying because these populations are close to valleys in the European Alps through which rapid spread to the rest of Europe is possible (Bertolino *et al.*, 2008). The effects of population mergers on genetic diversity and the subsequent effects on expansion should be considered in predictive models and squirrel management. Multiple nuclei should be sampled and their allelic compositions compared in the management of this and other invasions. Best-practice management should prioritize the prevention of mergers and should prevent further releases, as these augment diversity.

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SUPPORTING INFORMATION

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

Appendix S1 Habitat fragmentation.

Appendix S2 Genotyping methods.

Appendix S3 Hardy-Weinberg equilibrium, genotyping errors, and Bonferroni corrections.

Appendix S4 Support for the effective founder size formula.

Appendix S5 Additional discussion on founder size and expansion rate estimates.

Table S1 Multiplex set-ups and PCR conditions.

Table S2 Cluster distribution of the examined squirrels.

Table S3 Estimates of founder sizes.

Table S4 Migration rates per sampling location.

Figure S1 Population range expansion rate calculation for Piedmont, Italy.

Figure S2 Population range expansion rate calculation for Northern Ireland.

Figure S3 Population range expansion rate calculation for Northumberland.

Figure S4 Population range expansion rate calculation for East Anglia.

BIOSKETCH

This study is part of Anna Lisa Signorile's PhD thesis on genetic determinants of the expansion of grey squirrels in Europe. The main focus of the project was understanding the driving forces leading to squirrel success as invaders and the main spreading mechanisms.

Author contributions: All authors conceived the ideas; A.L.S. collected and analysed the data; J.W. supervised the genetic work and conceived the effective founder size formula; and A.L.S. and. D.C.R. led the writing.

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