The impact of past introductions on an iconic and economically important species, the red deer of Scotland

Sílvia Pérez-Espona[1,2*], Richard J. Hall[3], F. Javier Pérez-Barbería[2], Belinda C. Glass[3], Jamie F. Ward[3], Josephine M. Pemberton[1]

[1]Institute of Evolutionary Biology, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

[2]The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, UK

[3]AgResearch, Invermay Agricultural Centre, Puddle Alley, Private Bag 50034, Mosgiel 9053, New Zealand

*Corresponding author: Sílvia Pérez-Espona

E-mail: silvia.perez-espona@anglia.ac.uk; s.perezespona@googlemail.com

Running title: Effect of past introductions in Scottish red deer

Summary

The red deer (*Cervus elaphus*) is an iconic species in Scotland and, due to its value as a game species, an important element of the Scottish rural economy. The native status of this species is sometimes questioned because of many, recorded, introductions of non-native deer in the past in an attempt to improve trophy size. In this study, we assessed the impact of past introductions on the genetic make-up of Scottish red deer by genotyping at 15 microsatellite loci a large number of samples (n = 1,152), including mainland and island Scottish red deer and individuals from several putative external source populations used in introductions to improve trophy. Population structure and introgression assessment analyses revealed that the impact of introductions was weak in Highland red deer populations but more prominent on the islands, especially on those where current red deer populations are mostly or entirely

derived from introductions (Harris & Lewis, Arran and Rum). Frequent imports of Central-Eastern European red deer into English deer parks were reflected in the higher genetic introgression values found in some of the individuals collected in parks.

Keywords: *Cervus elaphus*, conservation, hybridization, introductions, introgression, microsatellites

Introduction

The red deer (*Cervus elaphus*) is one of the most economically important and widely distributed game species in Europe, with the largest continuous wild population found in Scotland (Apollonio et al. 2010). The red deer has had a continuous presence in Scotland since the end of the last ice age (c.11,000 years BP; Lister 1984), it is a key species for upland biodiversity and, due to its value as a game species, an important element in the Scottish rural economy (MacMillan & Philip 2008; Putman and Watson 2010). As for many other populations of red deer and game species in Europe, there is a long history of red deer populations being influenced by human activities since ancient times due to hunting, destruction of natural habitat, and introduction of exotic species (Pérez-Espona et al. 2009b; Zachos et al. 2011). Numerous introductions of non-native deer stock in an attempt to improve body and antler size of hunting trophies are known to have taken place in Scotland, in particular during the 19th Century (Whitehead 1960, 1964). Introductions of North American wapiti (*Cervus elaphus canadensis or Cervus canadensis*) to several Scottish estates have been documented, with over 30 individuals from a large wapiti herd introduced in Monymusk (Scotland) transferred in 1900 to Mamore Estate (Whitehead 1960, 1964). Matings between wapiti males and red deer females were encouraged by keeping individuals within enclosures (Whitehead 1960, 1964) and the two species (or subspecies) are known to

51 have successfully crossed in Britain (Winans 1913; Whitehead 1950) and in New Zealand
52 (Batcheler and McLennan 1977; Moore and Littejohn 1989). Similarly, Central European
53 (mainly German) and English park red deer were introduced directly to Scotland (Whitehead
54 1960, 1964). In a more indirect route, large Central-Eastern European red deer or wapiti
55 were crossed with British red deer in parks and later exported not only across Britain but also
56 to other European countries and far away countries such as New Zealand (Whitehead 1960,
57 1964). Translocations of red deer among Scottish localities in particular from the mainland to
58 the islands, are also reported and sometimes outnumbered those of non-Scottish deer
59 (Whitehead 1960, 1964).
60
61 Introductions of non-native deer stock to supplement native populations of red deer were a
62 common management practice across Europe (Apollonio et al. 2010; Linnell and Zachos
63 2011). However, in contrast to other countries where records of past common management
64 practices might not be readily available, the well documented history of introductions of non-
65 native deer into Scotland has raised questions regarding the native status of current Scottish
66 red deer populations. Previous studies assessing the impact of introductions and
67 translocations on the genetic make-up of Scottish red deer include several studies assessing
68 sika-red deer hybridisation on the Kintyre peninsula (Argyll) suggesting, that overall,
69 hybridization between the two species is rare except to the south and west of Loch Awe
70 (Abernethy 1994; Goodman et al. 1999; Senn and Pemberton 2009); a mitochondrial DNA
71 (mtDNA) survey on Rum (Nussey et al. 2006) which found that among a small number of
72 haplotypes present, one was highly divergent and related to Corsican deer (*Cervus elaphus*
73 *corsicanus*), the smallest subspecies of red deer and not documented to have been used for
74 trophy 'improvement' in Britain; a small study comprising a total of 69 red deer samples
75 collected in four Scottish mainland estates, two Scottish island estates and one English deer

76 park for which the lack of congruence between geographical and genetic structure (estimated
77 with microsatellite and mtDNA) was attributed to past management practices (Hmwe et al.
78 2006); a large (625 individuals) mtDNA survey within the Scottish Highlands that found no
79 haplotypes related to any exotic species or subspecies of deer, high haplotype diversity and a
80 pattern of population genetic structure largely concordant with geography, therefore
81 suggesting minimal impact of past management practices on matrilineal population genetic
82 structure (Pérez-Espona et al. 2009a); and a Y-chromosome survey at Mamore (where a
83 large number of wapiti were introduced in 1900, see above), two of its neighbouring estates
84 and one English deer park showing that all 149 individuals sequenced presented red deer Y-
85 chromosome haplotypes (Pérez-Espona et al. 2011).
86
87 Overall, with the exception of the sika-red deer hybridization studies in Argyll, previous
88 studies addressing the impact of introductions and translocations on the genetics of Scottish
89 red deer have either used uniparentally-inherited markers (Nussey et al. 2006; Pérez-Espona
90 et al. 2009a, 2011) or used a too small sample size of Scottish and English deer park to obtain
91 robust estimates of the effect of past management practice on Scottish red deer populations
92 (Hmwe et al. 2006). In this study, we genotyped 15 microsatellite loci in a large number of
93 samples collected on fourteen estates of the Scottish Highlands and seven west coast Scottish
94 islands, together with samples of North American wapiti, English park red deer, and Central
95 European red deer, the documented deer stock used for introductions to improve trophies in
96 Scotland, to assess how past introductions of non-native deer have affected the genetic make-
97 up of Scottish red deer.
98
99 We expect the effects of past management practices to be less apparent in areas where
100 animals were introduced with an aim to improve trophies, since this involved a small number

of individuals being introduced to a much larger 'native' population size. In contrast, we expect that the effects of introductions will be more notable on islands than mainland populations because introductions often served to repopulate diminished or extinct populations.

Methods

Sampling

The study comprised samples from a total of 1,152 red deer collected from the Scottish Highlands (n = 695), Scottish islands (n = 274), English deer parks (n = 109) and Central-Eastern Europe (n = 35), and samples from North American wapiti (n = 39) (see Fig. 1 & Table 1). Samples consisted of either ear or jaw muscle samples, or blood collected during legal hunting and culling programmes (Table 1).

DNA laboratory procedures

DNA extraction

Genomic DNA extractions from tissue were conducted using DNAace Spin Tissue Mini Kit (Bioline), DNEasy Tissue Kit^{TM} (QIAGEN) and the Chelex protocol (Walsh et al. 1991). DNA extraction from blood was conducted by using a standard Proteinase K/ethanol extraction procedure.

Microsatellite genotyping

All samples were genotyped at 15 microsatellite loci (FCB304, JP38, RT1, RT7, TGLA94, JP27, T26, T156, T193, T501, BM757, CSSM003, RM188, RT25, T268) following a previously developed high-throughput protocol (see details for redesigned primers and reactions in Pérez-Espona et al. 2008). Multiplex PCR products were run on an ABI 3730

capillary sequencer (Applied Biosystems) together with the internal size standard GeneScan 500 LIZ (Applied Biosystems). Genotypes from the Scottish Highland red deer were already available from previous studies (Pérez-Espona et al. 2008). To standardize genotyping, eight control samples included in the previous genotyping of Scottish Highland red deer were included in all the new genotyping plates. Fragment analysis was conducted using the software GENEMAPPER™ v. 3.0 (Applied Biosystems). All data scoring was conducted by the first author (SPE) in order to keep genotyping consistent across all samples.

Data analysis

Genetic diversity analyses

Deviations from Hardy–Weinberg equilibrium (HWE) and tests for linkage disequilibrium (LD) across all pairs of loci were conducted in FSTAT version 2.9.3 (Goudet 1995), with a strict Bonferroni correction applied for multiple comparisons ($\alpha = 0.05$). Null allele frequencies were calculated using CERVUS v. 2 (Marshall *et al.* 1998) using the algorithm from Summers & Amos (1997). FIS values for each locus and their statistical significant obtained with FSTAT. Genetic diversity measures such as mean number of alleles per population and observed (H_O) and expected heterozygosity (H_E) were calculated using MS TOOLS (Park 2001). Multilocus allelic richness and private allele richness correcting for sample size using a rarefaction procedure was estimated in ADZE v. 1.0 (Szpiech et al. 2008) for all populations, except for some of the samples from English deer park (EDP) and those from Central and Eastern Europe (GE, EE) as the number of individuals with complete genotypes was lower than for the other populations.

Population structure of Scottish red deer and introgression from non-native deer

The individual-based Bayesian clustering method implemented in the program STRUCTURE v. 2.1 (Pritchard et al. 2000) was used to assess population structure and genetic introgression from non-native stock. To assess population structure, the most likely number of populations (K) in our total data set was estimated by conducting five independent runs for K = 1-14, using a burn-in of 500,000 replications, 10^6 Markov chain Monte Carlo steps and assuming a model of admixture and a model of correlated frequencies among populations. In order to aid identification of the best K in our data set, we calculated Evanno's Δ*K* (Evanno *et al.* 2005) using the program STRUCTURE HARVESTER v. 0.6.8 (Earl *et al.* 2011). Bar plots of the results from STRUCTURE were conducted in DISTRUCT v. 1.1 (Rosenberg 2004). To assess introgression from non-native deer into Scottish red deer, we conducted further analyses in STRUCTURE using two different subsets of data: (i) The Scottish mainland red deer dataset was divided into three (West, Central, East) according to results obtained in this study (see Results); (ii) Each of the islands was considered a different data set except for Harris & Lewis which was divided into two (see Results). For each of these Scottish red deer data sets introgression from wapiti, English deer park or Central-Eastern European red deer was assessed in separate analyses. In a further analysis, possible introgression of wapiti and Central-Eastern European red deer into English park red deer was also evaluated. For each of the analyses we set the parameters as before, but set K = 2. Individuals' estimated proportion of membership (q) to the 'non-native' deer cluster and their 90% probability intervals were calculated to identify levels of introgression. The threshold value of $q \geq 0.1$ was used to detect admixed individuals as it has been shown to achieve efficient identification of admixture (Vahä and Primmer 2006).

Results

174 Genotyping of all individuals resulted in a matrix of genotypes 96.23% complete. Possible
175 incidence of null alleles was indicated for locus T156, as 8 out of the 26 populations were not
176 in HWE at this locus. However, ascribing the deviation of HWE in this locus to null alleles
177 was conservative. The critical P-value for detection of null alleles was P = 0.00013, but as the
178 software used to detect null alleles only gives 4 decimal points any populations with a P-
179 value = 0.0001 (the lowest p-value found in our data set) for a particular locus was
180 considered as a population with potential null alleles for that locus. A simulation-based study
181 showed that the inclusion of markers with potential null alleles, in particular at such low
182 frequencies as we have estimated, was not likely to affect analyses conducted in STRUCTURE
183 (Carlsson 2008). Furthermore, additional analyses conducted in STRUCTURE with the same
184 stringent parameters but excluding locus T156 gave the same results (see below); therefore,
185 the full data set was used in subsequent analyses.
186
187 Linkage disequilibrium was only detected between loci TGLA94 and T268 in Harris & Lewis
188 (HL-1). For four populations, Harris & Lewis (HL-1), Jura (JU), Arran (ARR) and one of the
189 Eastern European red deer population (EE), significant departures from HWE were suggested
190 (P = 0.0001; nominal P = 0.00013) with both populations presenting a low but significant
191 inbreeding coefficient $F_{IS} > 0.1$ (Table 2). Possible causes of departures from equilibrium in
192 these populations could be due to inbreeding, selection for or against particular alleles or the
193 presence of null alleles (amplification failure of a certain allele due to mutation in flanking
194 regions) at multiple particular loci (Selkoe and Toonen 2006). Inspection of the FIS values
195 locus by locus only suggested significant excess of homozygotes in locus T156 (P = 0.0001)
196 in JU and CSSM003 (P = 0.0001) in EE. Deviation from HWE of JU was suspected to be due
197 to inbreeding in this population, deviation from HWE equilibrium of EE is likely to be due to
198 Wahlund effect as samples included in this population were collected from distant localities.

Further analyses in STRUCTURE with the same stringent parameter settings to evaluate the effect of including JU in our STRUCTURE analyses revealed the same results (see below).

Genetic diversity

Genetic diversity indices per population and locus are summarised in Table S1, Table S2 and Fig. S1 (Supplementary material). The wapiti and English deer park populations had lower values of allelic richness than Scottish red deer, with the exception of HL1 which presented the lowest allelic richness of the studied populations (Fig S1a). However, wapiti presented a much larger proportion of private alleles, 60-70% higher, than the other red deer populations (Fig S1b).

Population structure of Scottish red deer and introgression of foreign deer stock

The analyses conducted in STRUCTURE revealed that the most likely number of clusters (genetic populations) increased when increasing the value of K (Fig. S2a). Calculations of Evanno's ΔK indicated the highest peak at K = 7 followed by another main peak at K = 10 (Fig S2b). Differences in clustering patterns between K = 7 and K = 10 were that samples from Arran and Rum clustered with Central-Eastern European red deer samples (GE-EE) at K = 7 but were further differentiated as a distinct cluster at K = 10; samples collected on Jura, Islay, Mull, South Uist and samples labelled as HL2 presented similarities to mainland populations at K = 7 but were further differentiated as a distinct cluster at K = 10; and increased differentiation at K = 10 of the highly admixed samples collected in the central part of our study area in the Scottish Highlands (Fig. 2). Wapiti, Central-Eastern European red deer and English park red deer formed three distinct clusters. The pattern of genetic structure found on the islands was more complex than that of the mainland, the latter agreeing with the

presence of landscape features within the study area (see Pérez-Espona et al. 2008). Some of the islands clustered together (Jura, Islay, Mull, South Uist and HL2; considering the admixture pattern, samples labelled as HL2 are likely to have been collected in Mull) but Harris & Lewis (HL1), Arran and Rum presented little admixture and formed two distinct clusters. Further analyses conducted in STRUCTURE with the same stringent parameters but excluding locus T156 were in agreement with the analyses conducted with the full dataset (see supplementary material). The likelihood of K increased with the number of K and started to plateau around K = 7-8 (Fig. S3a). Results from Evanno's delta K were as those obtained with the whole dataset with peaks found for the same K but with the highest peak found at K = 5 (Fig. S3b). Patterns of structure were practically identical to those found with the full data set with the only difference being that differentiation of RU from GE-EE occurring at K = 12 instead of K = 10 (Fig. S3c). Analyses conducted in STRUCTURE with the same stringent parameters but excluding population JU did also agree with the results obtained using the full dataset. The likelihood of K increased with the number of K and started to plateau at K = 7-8 (Fig. S4a). Major peaks in Evanno's delta were found at K = 5 and K = 12 but patterns of population structure were exactly the same as those found with the full data set (Fig. S4b and S4c).

Genetic introgression analyses

Results from the introgression analyses are summarised in Fig. 3 and Table S3. Genetic introgression of wapiti into Scottish and English park red deer was found to be minimal, with only one English park individual presenting a $q > 0.1$ to the wapiti cluster with its 90% probability interval not spanning 0. All of the individuals from the mainland and islands with $q > 0.1$ belonging to the English deer park cluster had 90% probability intervals spanning 0 Regarding introgression of Central-Eastern European red deer, only one individual from the

mainland (GST) and three from the islands (one in Harris & Lewis, three in Jura) had $q > 0.1$ with 90% probability intervals not spanning 0. Eight English park red deer presented values of $q > 0.1$ to the Central-Eastern European red deer cluster with 90% probability intervals not spanning 0.

Discussion

Evidence of genetic introgression of wapiti and Centra-Easternl European red deer in English deer park populations

Deer parks in Britain are thought to have existed since the Roman occupation, with their numbers increasing during the Norman era (11th Century) when deer were kept within walls to provide hunting for early monarchs (Hingston 1988). However, it was during Victorian times (19th Century) that the objective of holding deer for aesthetic reasons (and the associated selection for larger trophies) led to the greatest number of deer parks (Whitehead 1964; Hingston 1988). The number of parks greatly decreased after the outbreak of World War I, only to recover by the 1970s when demand for venison in Germany, that could not be provided domestically, was covered by exports of British venison (Hingston 1988). Although a good number of deer parks across Britain still exist nowadays, only about 60 of them have red deer on their grounds and about 30 parks contain a red deer population consisting of more than 70 individuals (Hingston 1988). Imports of Central European red deer, and to a lesser extent wapiti, into English deer parks have been a common practice in an attempt improve body and antler size. Deer from English parks were not only exported to other British localities but were also exported to other countries within and outside Europe (Whitehead 1960, 1964). These common management practices conducted in deer parks were apparent in our study from the particularly high levels of introgression of Central-Eastern European red deer detected with our panel of markers in English park red deer (7.4% of the individuals

sampled were introgressed, some of them presenting q> 0.5) and the detection, albeit at a low level, of wapiti introgression,.

Effect of past management practices on the genetic make-up of Scottish mainland red deer

Levels of genetic diversity (allelic richness) of Scottish red deer were higher in the mainland than in the island populations, with the exception of the island of Mull for which values were similar to those found in the mainland. The patterns of population structure found in mainland populations were in agreement with the location of major landscape features in the study area, with sea lochs, mountain slopes and roads acting as barriers for gene flow and inland lochs as facilitators of gene flow (Pérez-Espona et al. 2008). Genetic introgression of wapiti in Scottish Highland red deer populations assessed with our panel of markers was negligible even on the estate of Mamore, where a relatively large number of wapiti were introduced in the past (Whitehead 1960, 1964). This result coupled with the lack of wapiti mtDNA and Y chromosome sequences found in previous studies (Pérez-Espona et al. 2009a, 2011), gives further support for a low impact of wapiti introductions on the genetic make-up of Scottish Highland red deer. Effective wapiti introductions might have been hindered by high mortality of wapiti in Scotland, as this species has been reported to be susceptible to develop lung disease and foot malformation when exposed to the British climate (Winans 1913). Lower reproductive success of wapiti, due to the more aggressive behaviour of red deer males during the breeding season (Asher et al. 2000; B. Banwell and S. Burnett pers. comm.) or lower fecundity and survival of hybrid descendants (Asher et al. 2005), could also explain unsuccessful past wapiti introductions.

Genetic introgression of English park and Central-Eastern European red deer into Scottish Highland red deer was also found to be low with our panel of markers; only one individual

299 confirmed to be introgressed with Central-Eastern European red deer. Introductions of red
300 deer from Continental Europe (or admixed individuals) into the Scottish mainland via
301 imports from English parks are well documented; however, these generally involved the
302 import of a small number of individuals over decades (Whitehead 1960, 1964). The low
303 number of individuals introduced, relative to local population sizes, coupled with a possible
304 reduced ability to survive the harsher environmental conditions in Scotland could explain the
305 negligible effect of introductions on the genetic make-up of local larger red deer populations
306 using our marker set.
307
308 *Effect of past management practices on the genetic make-up of Scottish island red deer*
309 The impact of past management practices were found to be stronger on the Scottish islands
310 than on the mainland, as reflected by the more complex population structure and the higher
311 levels of Central-Eastern European red deer introgression observed. Mull, Jura, Islay, South
312 Uist, and individuals labelled as HL2 (but likely to have been collected in Mull) presented a
313 pattern of population genetic structure overall concordant with geography and their proximity
314 to the Scottish mainland. Red deer are known (Jura and Mull) or thought (Islay) to have
315 inhabited these islands since prehistoric times and to have maintained large populations until
316 the 18th-19th Centuries, when overhunting and habitat destruction caused large declines in red
317 deer populations. Measures to reduce hunting on the islands and imports of red deer mainly
318 from the mainland, and less often from English deer parks, allowed the recovery of the red
319 deer populations on these islands (Whitehead 1960). South Uist held a large population of red
320 deer in the past but it is thought to have become extinct by the end of the 18th Century
321 (Whithead 1960). Introductions of red deer in South Uist occurred in more recent times when
322 individuals were introduced from the Scottish mainland during 1970-1980s (Carne 2000).
323 Although samples collected from this island were too limited in our study to reach any

conclusions, the observed levels of admixture on this island and the lower divergence of South Uist red deer genetic make-up from other islands geographically closer to the mainland could be explained by the relatively recent nature of the introductions on this island (Nei et al. 1975).

Harris & Lewis, Arran and Rum presented levels of population structure that greatly differed from the mainland and the other islands included in our study. Archaeological studies have confirmed the presence of red deer on Harris & Lewis during prehistoric times but, due to the large distance of this island from the mainland, it is likely that red deer were introduced by humans even if in remote times. Similar to other islands, records confirm the abundance of red deer in Harris & Lewis until the end of the 18th Century and a later steep decline of the population (Whitehead 1964). Introductions of Scottish mainland red deer to re-establish the population and to improve hunting trophy size on Harris & Lewis are documented, although considered to have been largely unsuccessful as a result of the harsh environment on the island (Carne 2000); habitat quality has been shown to be an important factor associated with successful introductions (Griffith et al. 1989; Wolf et al. 1996). Individuals from Harris & Lewis (HL1) presented no admixture, formed a distinct cluster from other populations and presented the lowest allelic richness values among all Scottish red deer populations. This pattern of population structure and genetic diversity found in Harris & Lewis agrees with the theoretical expectations of loss of genetic diversity and increase genetic differentiation in populations after undergoing a strong bottleneck (Keller et al. 2001; Frankham et al. 2009). The genetic variation of the original red deer population of Harris & Lewis, however, is likely to have been low since colonization of the island, as only a few animals might have been able to be transported to this remote island. Therefore, founder effects and later populations bottlenecks coupled with the geographical isolation of Harris & Lewis from the

mainland would explain the divergence and low genetic diversity of its current red deer population. Genetic studies using DNA from prehistoric remains would be crucial to obtain further insights into the original level of genetic diversity of red deer populations in Harris & Lewis.

The distinctness and little admixture observed in the Rum and Arran red deer populations is explained by the fact that these populations originated from introductions of a relatively small number of individuals from the source populations, including deer parks, after the extinction (or near extinction) of their original populations (Whitehead 1960; Love 2001) and the subsequent stronger effects of genetic drift of these small populations leading to their divergence (Whitlock and McCauley 1990). The higher genetic diversity found in Arran and Rum, in comparison to Harris & Lewis, can be attributed to the known diverse genetic background of individuals used in the different introduction events undertaken to repopulate both islands (Tallmon et al. 2004). Despite the important role of English deer parks in introductions of red deer on Arran and Rum, the level of genetic introgression from English deer park (or Central European red deer) on these islands assessed with our panel of markers was low. This could be explained by a rapid growth of the islands' populations and strong divergence from the source population since the reintroductions (Caughley et al. 1994; Zenger et al. 2003).

Conclusions

The well-documented introductions of non-native deer stock into Scotland have raised questions regarding the native status of current Scottish red deer populations, the largest continuous population of red deer in Europe. This study represents the first attempt to assess the effect of introductions on the genetic status of Scottish red deer using a large number of

samples and bi-parentally inherited microsatellite markers. Our results gave further support to previous studies using uni-parentally markers (mtDNA or Y-chromosome markers) in which a low influence of past introductions was indicated for red deer populations in the Scottish Highlands. Results from this study provide further evidence of a strong influence of human management on some of the Scottish islands, in particular in Harris & Lewis, where the colonisation of the island by red deer is likely to have been conducted by man (even if in remote times), and Arran and Rum where original populations were drive to (o near to) extinction and later repopulated with stock originating partly from English deer parks. Introductions of Central-Eastern European red deer, and to a lesser extent wapiti, into English deer parks as a management practice to improve trophy size was reflected by the high levels of introgression presented by some of individuals collected in parks.

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555 **Figure legends**

556

557 **Figure 1.** Sampling sites in Scotland used in this study. See Table 1 for acronyms.

558

559 **Figure 2.** Plot of the estimates of q (estimated membership coefficient for each individual)
560 to each cluster for K = 7 and 10. Each vertical line represents an individual and is broken into
561 K coloured segments of length proportional to membership of each of the inferred clusters
562 (see methods).

563

564 **Figure 3.** Distribution of q values per population assessing: a) introgression of wapiti in
565 Scottish and English park red deer populations; b) introgression of English park deer in
566 Scottish red deer populations, c) introgression of Central-Eastern European red deer into
567 Scottish and English park red deer populations. Concentric circles indicate those individuals
568 for which the 90% probability interval of q-values did not overlap zero or 1.

569