Genetic diversity analysis of beavers (*Castor fiber*) in England

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Project details

Foreword

Natural England regularly commissions a range of reports from external contractors to provide evidence and advice to assist in delivering its duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

This report was commissioned to inform Natural England's advice to government on the approach to beaver reintroduction in England. One aspect of this relates to consideration of the best founding individuals for a reintroduction.

Although species reintroductions are a key conservation tool used to help restore species populations and/or ecosystem functions, genetic management needs to be an important consideration of any reintroduction strategy in line with the IUCN guidelines (IUCN and SSC 2013)[1] and the Reintroduction and other conservation translocations: code and guidance for England (Defra, 2021)[2]

The genetic composition of founder individuals for reintroduction projects can affect reintroduction success. Beaver reintroduction is a topic of increasing interest in England, but historically re-introductions of this species across Great Britain has been uncoordinated resulting in disjointed populations of varying status. In order to maximise the success of beaver reintroduction into England, consideration needs to be made of genetic aspects that will increase the likelihood of success.

This report investigates the genetic diversity in beavers currently present in England, both free-living and in enclosures, with a view to inform best practice for future reintroductions and management.

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[1] IUCN and SSC (2013). Guidelines for Reintroductions and Other Conservation Translocations. Gland, Switzerland, IUCN Species Survival Commission. **Version 1.0**.

[2] Defra (2021). Code and Good Practice Guidance for Reintroductions and Conservation Translocations in England. Department for Environment, Food and Rural Affairs, London. Reintroductions and other conservation translocations: code and guidance for England (publishing.service.gov.uk)

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Keywords

Eurasian beavers (*Castor fiber*), reintroduction, genetic diversity

Further information

This report can be downloaded from the Natural England Access to Evidence Catalogue. For information on Natural England publications contact the Natural England Enquiry Service on 0300 060 3900 or e-mail enquiries@naturalengland.org.uk.

Executive summary

Background

The Eurasian beaver (*Castor fiber*) was once widespread across mainland Europe and Britain but populations declined primarily due to beavers being hunted for their fur and other products. As a result, this species went extinct in Britain sometime between the 12th and 16th centuries. Some European mainland populations survived but in drastically reduced numbers. Since the 20th century, mainland European populations have begun to recover and some populations have been successfully reintroduced into areas where beavers had gone extinct.

After being extinct in Britain for over 400 years beavers are now found across two main locations in Scotland. A population was recently reintroduced into Knapdale, on the West coast of Scotland from Telemark in Norway through the Scottish Beaver Trial (2009 - 2014) which was the first licensed reintroduction of beavers into Britain. Another population of wild beavers was discovered in the River Tay catchment (Tayside) in 2006, but this population was comprised of unauthorised releases and/or individuals that had escaped from enclosures. Extensive studies were conducted to examine the viability of the population before the decision was made that the Tayside population could remain. Between 2017 and 2020, 16 beavers were translocated from Tayside to Knapdale to try and improve the genetic diversity in the Knapdale population.

In 2013, Natural England became aware of a population of wild beavers living on the River Otter, Devon. The origin of these individuals is unknown, but it is suspected that, similar to the Tayside beavers, they originated from unlicensed releases and/or individuals that have escaped from enclosures. After extensive studies of suitability, it was decided that the population in the River Otter, Devon be allowed to remain in an attempt to establish a viable, healthy population. The River Otter Beaver Trial is now the first licensed non-enclosure reintroduction trial of beavers into England.

Subsequent to individuals being discovered in the River Otter, beavers have also been found in the wild in eight counties in England, again likely to be the result of escapees from enclosures or unlicensed releases. Beavers are also being kept under license within fenced enclosures in 17 locations across England. The source of authorised translocations of beavers into England has been via imports from Europe as well as beavers being translocated from the Tayside population in Scotland.

As part of a data gathering exercise, Natural England has commissioned a study to investigate the underlying genetic diversity of beavers living in England. Genetic diversity is important for long-term population persistence and thus is a key component of a successful management strategy. This report outlines the genetic analysis that was undertaken on available beaver samples from England. Sample availability for beavers living in England is limited so this report will focus on: 1)

individuals that were licensed for translocation from Tayside, Scotland to multiple enclosures within England, 2) individuals living on the River Otter, Devon as part of the licensed reintroduction trial, 3) individuals in Devon not associated with the River Otter Trial, and 4) individuals (free-living and in enclosures) in Kent.

For each population and enclosure that samples were available for we estimated genetic diversity, and relatedness between individuals. We also compared genetic diversity and relatedness in each population and enclosure to established populations in Scotland, and across Europe. Based on these data, we discuss preliminary recommendations for future management strategies. Due to the limited number of samples available these results and recommendations are preliminary and should be interpreted with caution. Further sampling and additional studies will be required to provide a better understanding of the genetic diversity of beavers across England.

Key Points

- Beavers are currently residing in multiple locations across at least eight counties in England where some of these beavers are free-living and others are kept in enclosures.
- When taken as a country-wide metapopulation, the beavers currently residing in England have genetic diversity comparable to Scottish and European populations. These individuals have shown ancestry from four of the known European fur-trade refugia (Norway, France, Russia:Voronezh, and Belarus). It is likely that this diversity and mixed ancestry has been largely introduced to England by beavers originating from the mixed populations in Southern Germany.
- No evidence for ancestry from the Germany: Elbe (Hesse) fur trade refugia was found within any beavers in Great Britain.
- Low levels of relatedness were observed within the beavers currently situated in England. A total of 14% of the beaver pairs examined were shown to be related to some degree but the majority of these can be attributed to the translocation of family units; a practice which is favoured for maintaining a high standard of animal welfare.
- Translocations from Tayside to populations and enclosures in England are currently ongoing but unless all beavers in England will be effectively managed as a metapopulation, with continued translocations between locations, we advise that a larger number of founders are reintroduced into England to maximise the available genetic diversity.
- Due to the extreme bottlenecks experienced by beaver populations across Europe in the preceding centuries, genetic diversity should be maximised by using founders from multiple

source populations. Where feasible, we recommend additional translocations into England from multiple European populations. The majority of the genetic diversity currently seen in England is from Southern Germany and possibly from an unsampled Eastern European population. Therefore, individuals from Hesse, Germany; Voronezh, Russia; Rhône, France; and Norwegian populations may offer the most novel additions to genetic diversity.

- A rapid increase in population size will maximise retention of genetic diversity from the released founders.
- In this report, the genetic diversity of beavers in England has been assessed on the presumption that all individuals will be managed as a metapopulation. Given the current fragmented distribution of beavers in England, and use of fenced enclosures, this would require ongoing human-mediated movement of individuals between locations. If this is not a feasible strategy, it is likely that most, if not all, populations of beavers in England will require genetic reinforcement via additional translocations.
- Ultimately, a wider range of samples from free-living beavers in England is needed for a robust assessment of available genetic diversity and to inform downstream management decisions.

Contents

Introduction

The Royal Zoological Society of Scotland's WildGenes laboratory has been commissioned by Natural England to conduct genetic diversity analyses on Eurasian beavers (*Castor fiber*) in England. The Royal Zoological Society of Scotland (RZSS) has been a major partner in the reintroduction of Eurasian beavers to Britain after a more than 400-year absence. This project, based at Knapdale in Argyll, was the first successful, authorised reintroduction of a mammal into the UK. RZSS expertise in animal husbandry, veterinary care, translocations, and conservation genetics were instrumental to the success of the Scottish Beaver Trial (Goodman et al., 2012; Rosell et al., 2012), Scottish Beavers Reinforcement Project (Dowse et al., 2020), and wider management of beavers in Scotland since 2009 (Senn et al., 2014; Campbell- Palmer et al., 2020). Here we use our previous genetic experience from working with beavers in Scotland to inform potential reintroduction and management plans in England.

Project background

The Eurasian beaver (*Castor fiber*) is a semi aquatic rodent and a former native species of Britain. After the last ice-age beavers occurred throughout Europe, including Great Britain. However, the species was widely exploited for fur and other products, and was driven to extinction across much of Europe by the 1900s. Beavers are thought to have disappeared from Great Britain between the 12th and 16th century, and by the early 20th century it is thought that only five isolated populations remained in Europe; in France, Germany, Norway, Belarus and Russia totalling about 1,200 animals (Halley et al., 2012). Since that time, beaver numbers have recovered throughout much of their former range in Europe through protective regimes, hunting regulation, active reintroductions and natural recolonisation.

In Great Britain an official trial reintroduction of beavers took place in Knapdale forest, mid-Argyll in Scotland from 2009 to 2014 (the Scottish Beaver Trial) with the translocation of beavers from Norway (Jones and Campbell-Palmer, 2014). The population in Knapdale was later reinforced through the Scottish Beavers Project (2017-2020), which aimed to boost numbers and genetic

diversity in Knapdale beavers by sourcing individuals from captive populations and translocating beavers from a population in Tayside, East Scotland (Dowse et al., 2020). The Knapdale population has persisted and is now believed to be at capacity, holding 20-30 beavers (Dowse et al., 2020).

Unlike Knapdale, the population in Tayside was not an authorised release but instead was comprised of unauthorised releases and/or individuals that had escaped from enclosures. These beavers are situated across parts of the Tay and Forth catchments (referred to as Tayside) in Perthshire, Scotland, with confirmed reports of their presence dating back to 2006. As the population in Tayside was not an authorised release a government consultation was undertaken to

determine how best to manage these beavers. Extensive studies were conducted to examine the viability of the population before the decision was made that the Tayside population could remain. Tayside is now the largest beaver population in Scotland (n = 954 beavers; range 602 – 1381, Campbell-Palmer et al., 2021).

In England, a population of breeding beavers was discovered on the River Otter in East Devon, in 2013. Their origins are unknown, but similar to the population in Tayside, it is believed that these individuals are the result of unlicensed releases and/or individuals that have escaped from enclosures. After extensive studies of suitability, it was decided that the population in the River Otter, Devon be allowed to remain in an attempt to establish a viable, healthy population. This population subsequently became the first licensed non-enclosure reintroduction trial in England and is under ongoing management.

Natural England is also aware of small numbers of beavers living in the wild in eight counties in England. A tributary of the River Tamar in Devon is thought to be home to a breeding population of Bavarian descent, which are likely to have escaped from a nearby enclosure. Other free-living populations of beavers in England include the River Stour in Kent, and additional unconfirmed reports of free-living beavers at sites in Kent, Herefordshire, Somerset and other parts of Devon. However, the number of beavers and breeding status in these locations are unknown.

As of 2021, there are 25 locations in England where beavers are kept within fenced enclosures where 17 sites are licensed and 8 sites are currently unlicensed (Heydon et al., 2021). This number has been increasing, but a new system of licensing has been introduced into England such that all new enclosed projects must apply for a license in advance. Some of the more recent translocations into enclosures in England have involved beavers from Tayside. Beavers in Scotland are managed as a European Protected Species but under strict circumstances lethal control licences can be issued by NatureScot for beavers that have come into conflict with landowners and where there are no other options for mitigation (see Management Framework for Beavers in Scotland). Where possible, and with the landowner's permission, individuals are translocated to enclosures to avoid dispatch under licence.

In response to the presence of beavers in England, Natural England is gathering advice to help inform the government's decision and future policy on the legal status, ecological benefits and impacts, future reintroduction and management of beavers more widely throughout England. To maximise the success of beaver reintroduction into England, genetic aspects that will increase the likelihood of success in the long term need to be considered. Genetic management is an important consideration of any reintroduction strategy and is outlined in the IUCN guidelines (IUCN 2013) as a necessary criteria that should be considered for any reintroduction.

Considering the above context, the aims of this report are to use currently available samples to:

- Estimate the genetic diversity, examine relatedness between individuals, and

determine the origin populations of beavers currently in England, both free-living and in enclosures.

- Compare the genetic diversity of beavers in England with beavers residing in established populations in Scotland, and the rest of Europe.
- Inform best practices for future reintroductions and management of beavers in England.

Work required

As per the original tender, to undertake genetic analysis of samples from:

1. Beavers moved to enclosures in England from Tayside in Scotland (n 12)
2. Beavers from the river otter in Devon (n 5[3])
3. Beavers from Devon in 2016 (n 4[4])
4. Beavers captured from the wild in Kent and subsequently held in captivity (n 3-4)
5. To analyse the results and put into perspective in relation to Scottish and European populations
6. To provide recommendations for a potential future reintroduction strategy

Details of the samples from England and the reference samples used for the genetic analysis are outlined in the sample summary section below. The sample numbers have changed from the proposed numbers outlined above due to the ongoing nature of the project. However, the number of samples available is still relatively limited and, as such, these results are preliminary. A considerable proportion of the individuals examined here are also currently kept in licensed enclosures and are not currently contributing to the available genetic diversity of wild beavers in England unless these populations are to be actively managed as a metapopulation. As such all results presented within this report should be considered with caution and all management decisions should acknowledge these limitations. Ultimately, further sampling of free- living beavers, from multiple locations, will be required to provide a more comprehensive understanding of the genetic diversity of beavers across England.

[3] Samples provided by Dr Róisín Campbell-Palmer

[4] Samples held by the Royal Zoological Society of Scotland

Sample summary

To achieve the aims set out in this project, genetic data was required from individuals living wild in Scotland, and representatives from populations in mainland Europe. RZSS already had access to a large amount of reference sample data, including samples from five European fur-trade refugia populations (Norway, Germany, France, Belarus, and Russia: Voronezh) (Halley et al., 2020; Senn et al., 2014) and the two established Scottish reintroduced populations (Knapdale and Tayside). A breakdown of sample numbers by population are outlined in Table 1.

Table 1. Samples identified for use as reference samples for comparisons in this study. The table indicates how many samples were received in total and the number of samples that yielded sufficient quality mtDNA and ddRAD sequences for downstream analysis.

Location	Origins	Subspecies classification*	Total samples evaluated	Samples used in analysis
Reference samples			**215**	**202**
Germany (Baden-Württemberg)	Reintroduced using beavers from France	*Castor fiber galliae*	6	6
Germany (Bavaria)	Reintroduced using mixed stock	*C. f. fiber, belorussicus, galliae*, & possibly *orientoeuropaeus*	25	24
Germany (Hesse)	Reintroduced using beavers from Elbe Fur trade refugia	*C. f. albicus*	13	13
Norway (Hedmark)	Expansion from Telemark	*C. f. fiber*	12	12
Norway (Telemark)	Fur trade refugia	*C. f. fiber*	28	27
France	Fur trade refugia	*C. f. galliae*	7	6
Belarus	Fur trade refugia	*C. f. belorussicus*	4	2
Russia	Fur trade refugia	*C. f. orientoeuropaeus*	10	10
Knapdale, Scotland	Reintroduced from Norway (reinforced with beavers from Tayside and of Bavarian origin)	Mixed	42	37

Location	Origins	Subspecies classification*	Total samples evaluated	Samples used in analysis
Tayside, Scotland	Escaped/illegal release ofbeavers with likely Bavarian origin	Mixed	68	65

*as summarised in Senn et al., 2014

Sample availability for beavers living in England is limited so this report will focus on: 1) individuals that were licensed for translocation from Tayside, Scotland to multiple enclosures within England, 2) individuals living on the River Otter, Devon as part of the licensed reintroduction trial, 3) individuals in Devon not associated with the River Otter Trial, and 4) individuals (free-living and in enclosures) in Kent.

Samples and corresponding field observations for beavers translocated from Tayside to England were provided to RZSS WildGenes by Dr Róisín Campbell-Palmer. Apart from Kent, all remaining individuals from England were sampled by RZSS vets, after trapping by Dr Róisín Campbell-Palmer under license from the Devon Wildlife Trust. Samples from Kent were provided by the Wildwood Trust. A breakdown of sample numbers by enclosure and population are outlined in Table 2.

Table 2. Samples identified for testing in this study. The table indicates how many samples were receivedin total and the number of samples that yielded sufficient quality mtDNA and ddRAD sequences for downstream analysis.

Location	Origins	Subspecies classification	Total samples received	Samples used in analysis
English samples to be tested			**42**	**41**
Cheshire	Tayside	Mixed	2	2
Cornwall	Tayside	Mixed	3	3
Cumbria	Tayside	Mixed	3	3
Devon	Tayside & unknown	Mixed	23	22
Gloucestershire	Tayside	Mixed	2	2
Kent	Tayside, Bavaria & unknown	Mixed	4	4
Norfolk	Tayside	Mixed	4	4
West Sussex	Tayside	Mixed	1	1

Methods

Laboratory procedures

Briefly, DNA was extracted from all available samples from Scottish and European reference populations, and all individuals currently in England as outlined in Table 1 and Table 2, respectively. Samples that successfully produced high quality and quantity DNA were used for downstream data production using two different techniques (mtDNA and ddRAD). All data produced were subject to quality controls to remove poor quality data and ensure robust downstream analysis.

Data analysis

The first technique focused on using mitochondrial DNA (mtDNA). This is a small section of maternally inherited DNA that can provide low resolution information about ancestry down the maternal line. As the origins of the samples within this report are largely unknown, mtDNA analysis can provide an initial assessment of which refugia population these individuals may have originated from.

We also used a technique to analyse genetic diversity more widely across nuclear DNA, which makes up the majority of an individual's genome and is inherited both maternally and paternally. We used a double- digest restriction-site associated DNA (ddRAD) technique that allows for hundreds to thousands of variable regions to be compared between individuals, providing higher resolution analyses.

This technique allows us to measure the genetic diversity of populations using a selection of diversity measures. We looked at three separate genetic diversity measures: observed heterozygosity (HO), where larger values signify higher genetic variation; proportion of polymorphic markers (PN), where higher values signify higher potential to adapt to future change; and an estimate of inbreeding (fhat3), where higher values signify that individuals are more inbred. The diversity measures were compared across enclosures and populations in England, and then compared to reference populations in Scotland and mainland Europe.

We were also able to estimate the degree of relatedness between pairs of individuals in each enclosure and population. For individuals that were translocated from Tayside to England we used this analysis to identify whether there are any potential close relations that were translocated together. While measures are taken to avoid potential breeding between closely related individuals, management decisions also have to consider the biology of the animals. Beavers are highly social, so relatives are often purposefully translocated together to maintain family units, which are essential to their welfare, especially when offspring are young. In some

cases, older siblings can be translocated together as a family unit where same-sex pairings would show high pairwise relatedness, but would not result in breeding between closely related individuals. As such, the presence of closely related individuals doesn't always indicate a potential for inbreeding. We compared our pairwise relatedness results to field observations and management decisions provided by Dr Róisín Campbell-Palmer.

In addition, we also used this data to determine the likely origin population for all individuals. While the mtDNA gives us an initial assessment of which refugia population an individual originated from, the ddRAD data allows us to examine more recent migration patterns.

A full, detailed description of the methods used, quality controls, and the analyses undertaken is available in Appendix I.

Results

Beavers moved to enclosures in England from Tayside in Scotland

We received a total of 28 samples from individuals that had been translocated from Tayside, Scotland into enclosures in England. Individuals were transferred to enclosures in the following counties; Cheshire (n = 2), Cornwall (n = 3), Cumbria (n = 3), Devon (n = 12), Gloucestershire (n = 2), Kent (n = 1), Norfolk (n = 4), and West Sussex (n = 1).

Firstly, we compared these translocated individuals to the Tayside reference samples collected from across the river catchment (n = 65) to examine whether the genetic diversity known to be present in Tayside has been captured in the translocations to England (Figure 1). The observed heterozygosity was higher in the translocated individuals (HO = 0.255) than the Tayside reference population (HO = 0.251) suggesting there may be a greater potential to adapt to change across the translocated individuals than the Tayside reference. The estimate of inbreeding was lower in the translocated individuals (fhat3 = -0.008) compared to Tayside (fhat3 = 0.018) suggesting the translocated individuals are less inbred than the base level of inbreeding seen within Tayside. Conversely, the proportion of polymorphic markers was lower in the translocated individuals (PN

= 0.787) than the Tayside reference population (PN = 0.829) suggesting that more genetic variability is present in Tayside than was captured in the translocated individuals. Notwithstanding, the differences in absolute values for all these measures are small and, as such, the translocated individuals are a reasonable representation of the known genetic variation available in Tayside.

As these populations are currently not managed as a single, metapopulation, we also calculated these values for each enclosure and population separately. These genetic diversity measurements are available in Appendix II Figure 1 but should be treated with caution; the number of samples tested at each location was extremely small (between n = 1 and n = 12) making these analyses less robust.

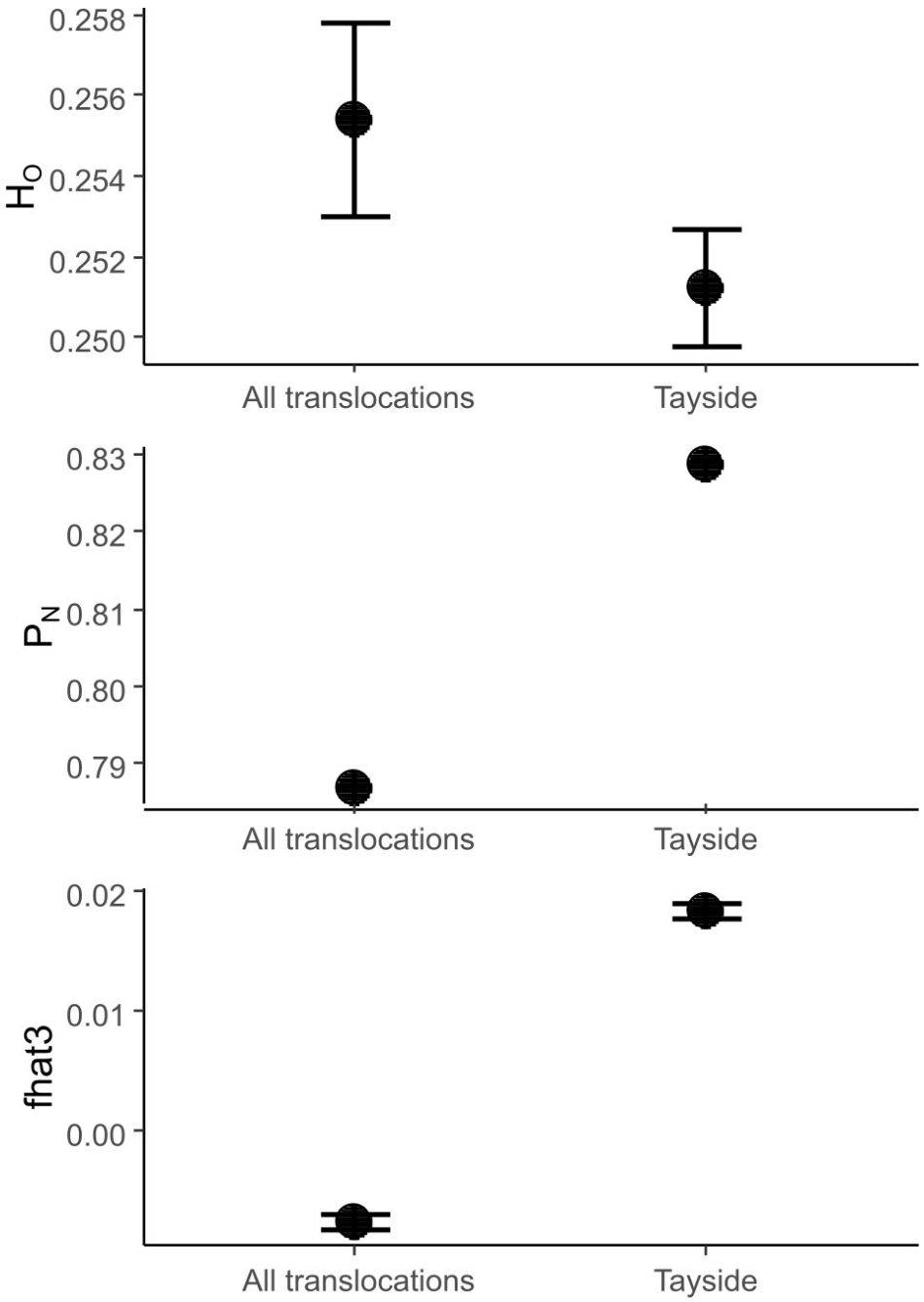

Figure 1. Graph of multiple genetic diversity statistics for all translocated individuals, and a Tayside reference population. H_O: observed heterozygosity, P_N: proportion of polymorphic markers, fhat3: average inbreeding coefficient. Bars represent the 95% confidence interval.

Secondly, we estimated the pairwise relatedness between the translocated individuals to identify whether there are any close relatives that have been translocated together, and we compared our results to field observations. Pairwise relatedness values were plotted as a heatmap (Figure 2) and all values are available in Appendix III Table 1 alongside the enclosure location that each individual was translocated to.

We calculated the average relatedness (r = 0.019) for the Tayside reference population (n = 65) as a baseline measurement for expected levels of relatedness for the translocated individuals. Populations with a higher average relatedness than Tayside were Cornwall (n = 3, r = 0.115), Devon (n = 12, r = 0.061), and Norfolk (n = 4, r = 0.150). These populations may therefore contain closely related individuals.

Within Cornwall two individuals (BEV814 and BEV816) were identified as being potentially related (r = 0.397). This agreed with field observations which suspected the two individuals to be sisters (trapped as kits) due to their observed behaviour and the fact they were caught in the same trap in an established territory (pers. comm. Dr Róisín Campbell-Palmer). The decision was taken to translocate the individuals together as a family unit.

Within Devon there were 20 instances of close relations being identified but it is important to note that these are split across four populations and enclosures. Individuals BEV803 and BEV805 were identified as being highly related (r = 0.492) and this reflects field observations that identified BEV805 as the mother of BEV803 (pers. comm. Dr Róisín Campbell-Palmer). Field observations also identified BEV811 and BEV812 as siblings which agreed with the relatedness analysis (r = 0.438), as well as identifying BEV812 and BEV813 as suspected relations again agreeing with the relatedness analysis (r = 0.144). Again, in both these cases the decision had been made to translocate family units together.

Within Norfolk three instances of close relations were identified. BEV809 was identified as a potential relation to BEV821 (r = 0.423), and BEV823 (r = 0.359), but there were no field observations to corroborate this assertion. The genetic data also suggested that BEV821 is related to BEV823 (r = 0.308), which matches field observations that noted them as brothers (pers. comm. Dr Róisín Campbell-Palmer). As multiple pairs were being released into the enclosure in Norfolk the decision was taken to release the brothers together as it was believed there would be less aggression between brothers than two unrelated males.

Further instances of potential relations were noted between individuals translocated to different enclosures and populations in England. While this is not of immediate concern, if all beavers currently in England are to be managed as a metapopulation the relatedness values between all individuals must be considered carefully. Full details of the field observations and the corresponding genetic relatedness scores are available in Appendix III Table 5.

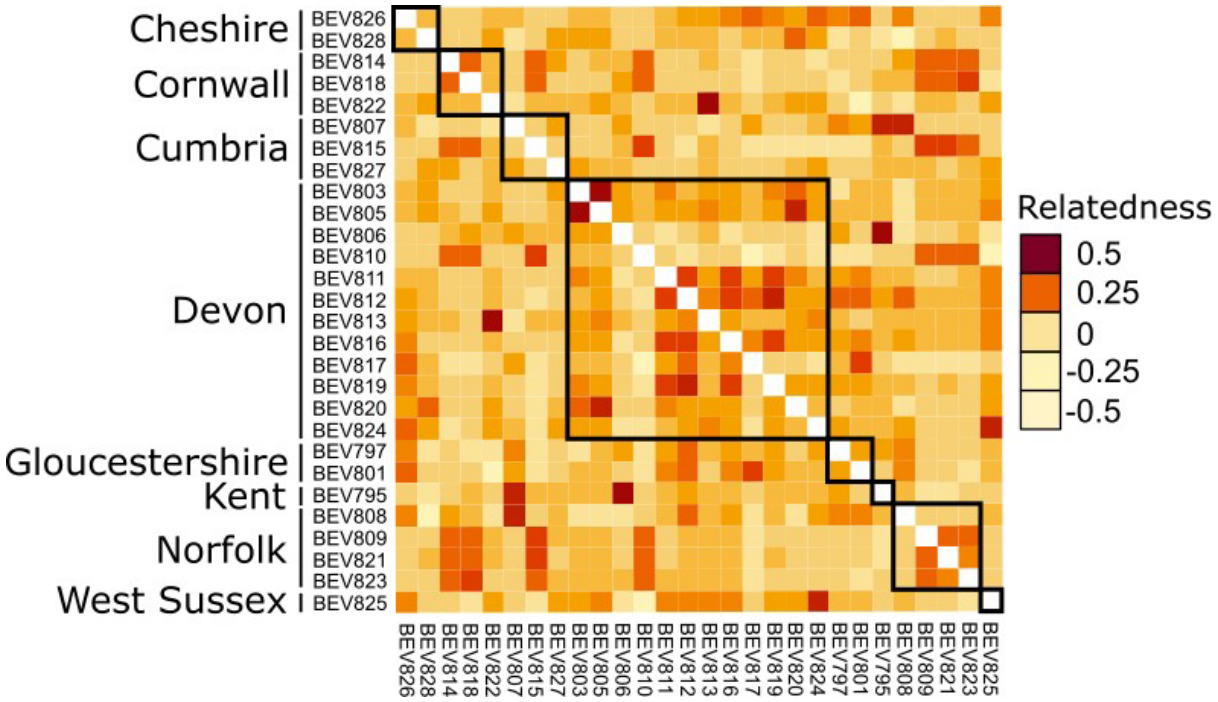

Figure 2. Heatmap of pairwise relatedness values between each pair of individuals. The darker the square the higher level of relatedness between those two individuals. Populations are grouped together in black boxes.

Thirdly, we compared these translocated individuals to reference European populations to determine which European refugia they may have originated from. Assignment analysis suggested that all individuals had some ancestry originating from either the Bavaria or Baden-Württemberg populations in Germany (Table 3). This was supported by our STRUCTURE analysis (Figure 13), which also suggested that these individuals likely had ancestry from Bavaria or Baden- Württemberg. However, the STRUCTURE analysis also revealed an additional genetic signature in the Tayside population that was more closely aligned to the Belarus reference individuals (see section 5 for further discussion). Evidence of this genetic signature was found in the majority of beavers transferred from Tayside to enclosures in England.

It is important to note that the overall support for population assignment varied greatly among samples. Some samples had very high support for their population assignment (e.g. BEV826 in Cheshire had a probability (P) value of 0.993 out of a maximum 1) while others had very low support (e.g. BEV814 in Cornwall had a P value of 0.060). Individuals with low support for population assignments are likely of mixed origin or have ancestry from a currently unsampled reference population. This is also supported by our STRUCTURE analysis (Figure 13) which showed the translocated individuals to have a similar assignment profile to those from the

Bavaria and Baden-Württemberg populations, which are themselves, reintroduced populations of mixed origins (see Table 1 for details).

We also re-ran this analysis to include the Tayside and Knapdale populations as references. This resulted in all individuals having the highest probability of originating from the Tayside population followed by the second and third highest probabilities of originating from the Bavaria or Baden-Württemberg populations in Germany. This is consistent with our expectations as all translocated beavers were moved from Tayside, and the Tayside population itself is largely comprised of individuals thought to originate from Bavaria and Baden-Württemberg (Campbell-Palmer et al., 2020).

Table 3. Proposed origin populations for the translocated individuals as determined by GeneClass2. P is the probability that an individual belongs to that population.

		GeneClass2 assignment			
Current population	**Individual**	**Assigned population 1**	**P**	**Assigned population 2**	**P**
Cheshire	BEV826	Germany (Bavaria)	0.993	Germany (Baden-Württemberg)	0.369
	BEV828	Germany (Bavaria)	0.156	Germany (Baden-Württemberg)	0.099
Cornwall	BEV814	Germany (Baden-Württemberg)	0.060	Germany (Bavaria)	0.018
	BEV818	Germany (Baden-Württemberg)	0.061	Germany (Bavaria)	0.023
	BEV822	Germany (Bavaria)	0.088	Germany (Baden-Württemberg)	0.081
Cumbria	BEV807	Germany (Baden-Württemberg)	0.105	Germany (Bavaria)	0.104
	BEV815	Germany (Baden-Württemberg)	0.041	Germany (Bavaria)	0.012
	BEV827	Germany (Bavaria)	0.138	Germany (Baden-Württemberg)	0.090
Devon	BEV803	Germany (Bavaria)	0.256	Germany (Baden-Württemberg)	0.146
	BEV805	Germany (Bavaria)	0.118	Germany (Baden-Württemberg)	0.109
	BEV806	Germany (Bavaria)	0.071	Germany (Baden-Württemberg)	0.050
	BEV810	Germany (Baden-Württemberg)	0.054	Germany (Bavaria)	0.035
	BEV811	Germany (Bavaria)	0.175	Germany (Baden-Württemberg)	0.161
	BEV812	Germany (Bavaria)	0.444	Germany (Baden-Württemberg)	0.228
	BEV813	Germany (Bavaria)	0.186	Germany (Baden-Württemberg)	0.136
	BEV816	Germany (Bavaria)	0.550	Germany (Baden-Württemberg)	0.250

		GeneClass2 assignment			
Current population	**Individual**	**Assigned population 1**	**P**	**Assigned population 2**	**P**
	BEV817	Germany (Bavaria)	0.747	Germany (Baden-Württemberg)	0.338
	BEV819	Germany (Bavaria)	0.384	Germany (Baden-Württemberg)	0.173
	BEV820	Germany (Bavaria)	0.389	Germany (Baden-Württemberg)	0.213
	BEV824	Germany (Bavaria)	0.371	Germany (Baden-Württemberg)	0.154
Gloucestershire	BEV797	Germany (Bavaria)	0.339	Germany (Baden-Württemberg)	0.212
	BEV801	Germany (Bavaria)	0.333	Germany (Baden-Württemberg)	0.252
Kent	BEV795	Germany (Bavaria)	0.088	Germany (Baden-Württemberg)	0.075
Norfolk	BEV808	Germany (Baden-Württemberg)	0.110	Germany (Bavaria)	0.103
	BEV809	Germany (Baden-Württemberg)	0.053	Germany (Bavaria)	0.017
	BEV821	Germany (Bavaria)	0.081	Germany (Baden-Württemberg)	0.080
	BEV823	Germany (Bavaria)	0.058	Germany (Baden-Württemberg)	0.052
West Sussex	BEV825	Germany (Bavaria)	0.206	Germany (Baden-Württemberg)	0.139

Beavers from the River Otter in Devon

We have access to samples from five individuals from the wild population of beavers in the River Otter, East Devon.

Firstly, we compared these River Otter individuals to Knapdale and Tayside reference populations to examine whether the genetic diversity in the River Otter population is comparable to the established Scottish populations (Figure 3). Genetic diversity in the River Otter, Devon population (HO = 0.236) is comparable to the Tayside reference population (HO = 0.251) and higher than the Knapdale reference population (HO = 0.153). However, it should be noted that the lower HO values reported for Knapdale is due to a lack of genetic mixing between the Norwegian and Bavarian origin beavers currently present there, which is expected to change over the coming years (Dowse et al., 2020). The proportion of polymorphic markers was low (PN = 0.425) compared to Tayside (PN = 0.829) and Knapdale (PN = 0.848). The inbreeding value in River Otter, Devon (fhat3 = -0.157) was also lower than Tayside (fhat3 = 0.018) and Knapdale (fhat3 = 0.216). It is important to note that all the values calculated for the River Otter, Devon population are likely to be inflated as the sample size (n = 5) is well below the standard minimum sample size (n = 20) required for these analyses.

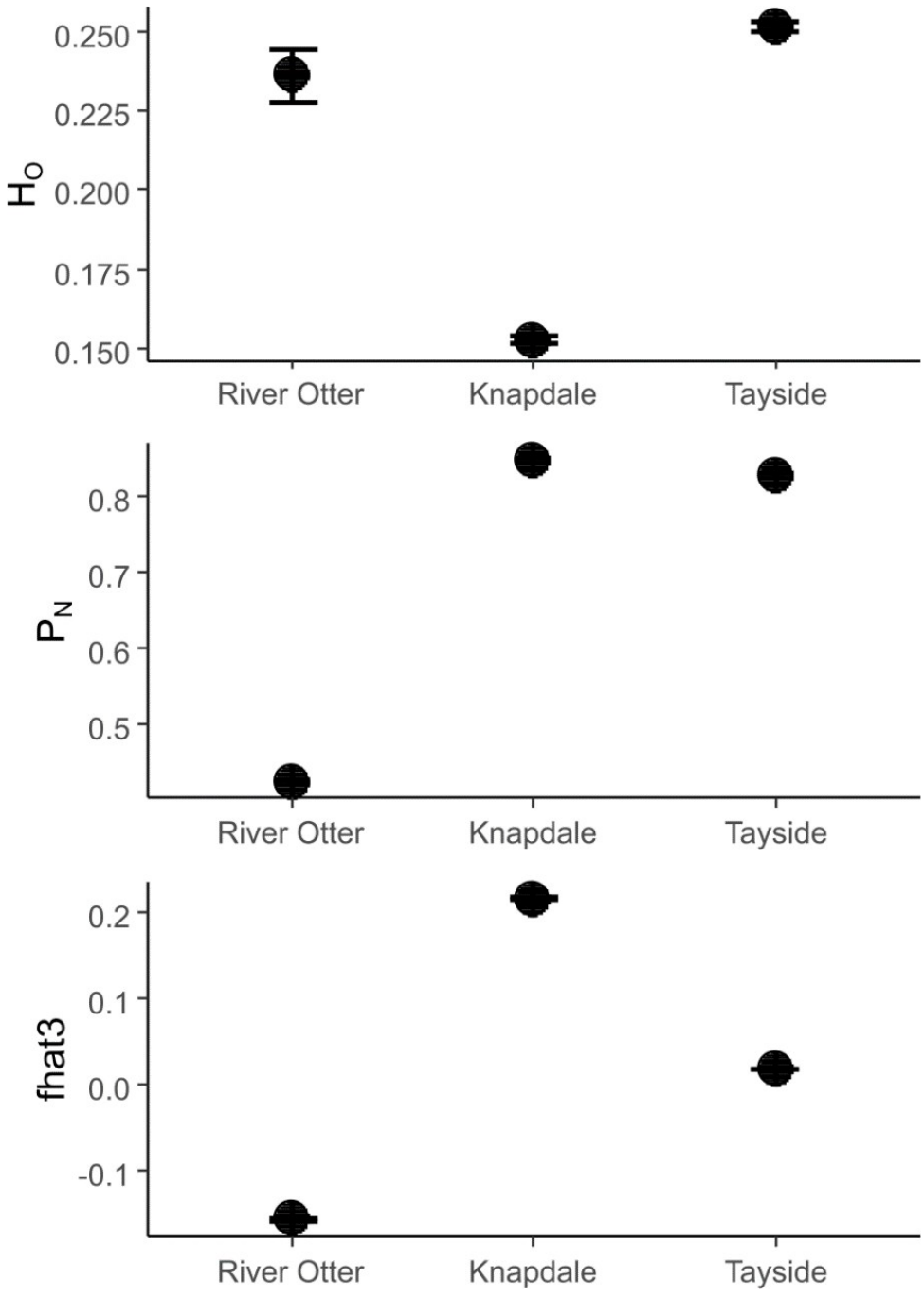

Figure 3. Graph of multiple genetic diversity statistics for the River Otter population, a Knapdale reference population, and a Tayside reference population. H_O: observed heterozygosity, P_N: proportion of polymorphic markers, fhat3: average inbreeding coefficient. Bars represent the 95% confidence interval.

Secondly, we examined the pairwise relatedness between the River Otter individuals to identify whether there are any close relatives in the population (Figure 4). The average relatedness across the River Otter population (n = 5) was relatively high (r = 0.480). All pairs of beavers were estimated to be close relatives. These results are consistent with previous analysis conducted on these individuals as part of the River Otter Beaver Trial (Campbell-Palmer et al., 2015). All relatedness values are available in Appendix III Table 2.

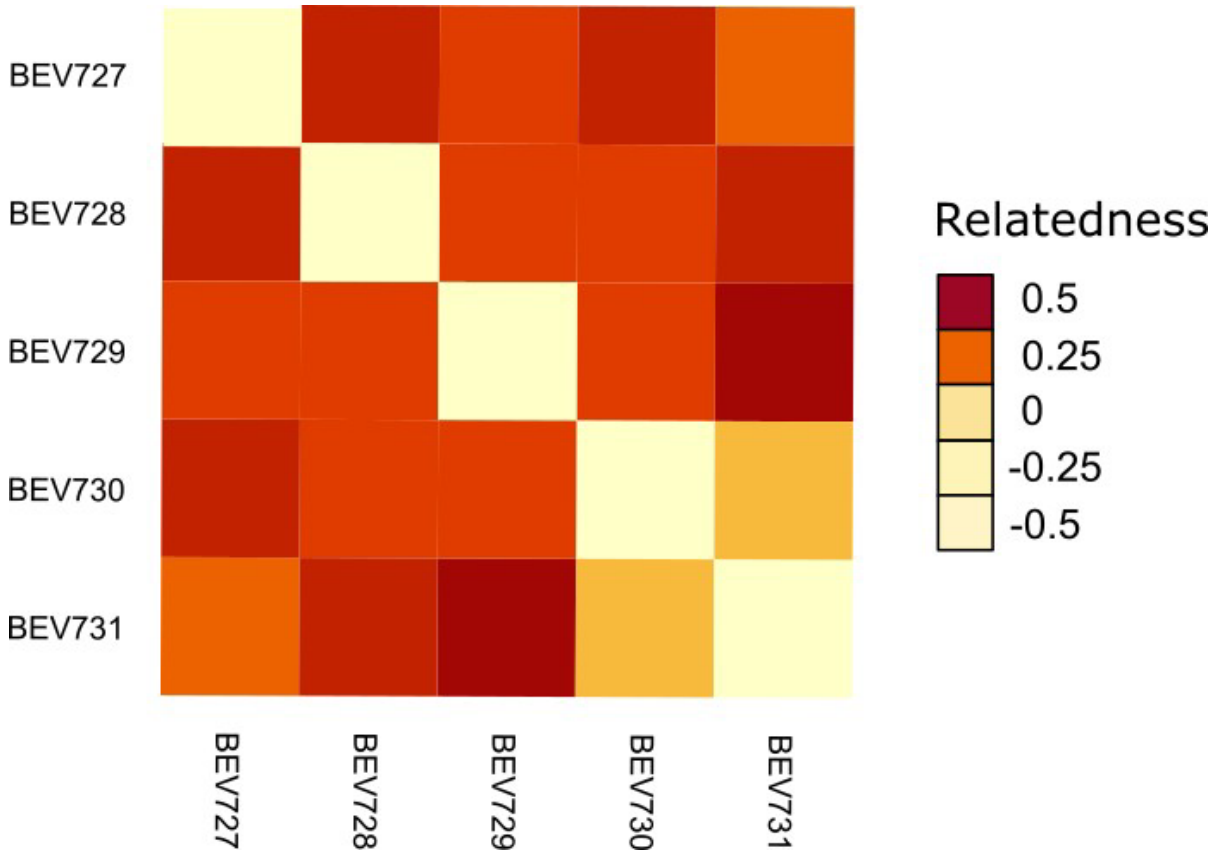

Figure 4. Heatmap of pairwise relatedness values between each pair of individuals. The darker the square the higher level of relatedness between those two individuals.

Thirdly, we compared these individuals to reference European populations to determine which European refugia they may have originated from (Table 4). Beavers from the River Otter, Devon were thought to be of Bavarian decent and all individuals examined here showed a high probability of originating from Bavaria, Germany. These results are consistent with previous analysis conducted on these individuals as part of the River Otter Beaver Trial (Campbell-Palmer et al., 2015).

Table 4. Proposed origin populations for the River Otter beavers as determined by GeneClass2. P is the probability that an individual belongs to that population.

	GeneClass2 assignment			
Individual	**Assigned population 1**	**P**	**Assigned population 2**	**P**
BEV727	Germany (Bavaria)	0.970	Germany (Baden-Württemberg)	0.413
BEV728	Germany (Bavaria)	0.951	Germany (Baden-Württemberg)	0.450
BEV729	Germany (Bavaria)	0.809	Germany (Baden-Württemberg)	0.444
BEV730	Germany (Bavaria)	0.975	Germany (Baden-Württemberg)	0.382
BEV731	Germany (Bavaria)	0.478	Germany (Baden-Württemberg)	0.245

Beavers from Devon

We received samples taken from a total of five individuals from various locations within Devon during 2016. These individuals were sampled from multiple locations, across a large geographical area and cannot be considered as a single, breeding population. Due to the limited number of individuals these samples were combined with the individuals from the River Otter, Devon (see section 2) to create a pre-translocation Devon metapopulation. We then combined this pre- translocation population with the individuals that were translocated from Tayside to Devon to create a post-translocation metapopulation. Ultimately, these values are based on the presumption that these individuals will be managed as a single metapopulation through ongoing translocations and, as such, these values should be considered with caution.

Firstly, we compared the pre-translocation metapopulation and post-translocation metapopulation to the Knapdale and Tayside reference populations to examine the effect of the translocated individuals on genetic diversity, and to examine whether the known genetic diversity across Devon is comparable to the established Scottish populations (Figure 5). Translocation has significantly improved the genetic diversity of beavers within Devon (pre-translocation HO = 0.225, post-translocation HO = 0.238) which is still lower, but comparable, to the Tayside reference population (HO = 0.251) and higher than the Knapdale reference population (HO = 0.153). Again, it should be noted that the lower HO values reported for Knapdale are due to a lack of genetic mixing between the Norwegian and Bavarian origin beavers which is expected to change over the coming years (Dowse et al., 2020). The translocations also improved the proportion of polymorphic markers from PN = 0.572 (pre-translocation) to PN = 0.757 (post-translocation), which is comparable to the Tayside (PN = 0.829) and Knapdale (PN = 0.848) reference populations.

The inbreeding value after the addition of translocated individuals (fhat3 = -0.013) remains similarly low to the inbreeding value before translocation (fhat3 = -0.067). Both of these values are lower than the Tayside (fhat3 = 0.018) and the Knapdale (fhat3 = 0.216) reference populations. It is important to treat the low inbreeding values with caution as these may be a statistical or sampling artefact. Specifically, inbreeding values are calculated based on the assumption that all individuals being tested as a population have had an equal opportunity to breed with other individuals in the population. Here we have effectively combined multiple groups of individuals that have not had the opportunity to breed together and this may be creating artificially low inbreeding values. Furthermore, a previous report on the River Otter, Devon individuals identified an inbred family (Campbell-Palmer et al., 2015), which may also be overrepresented in the data.

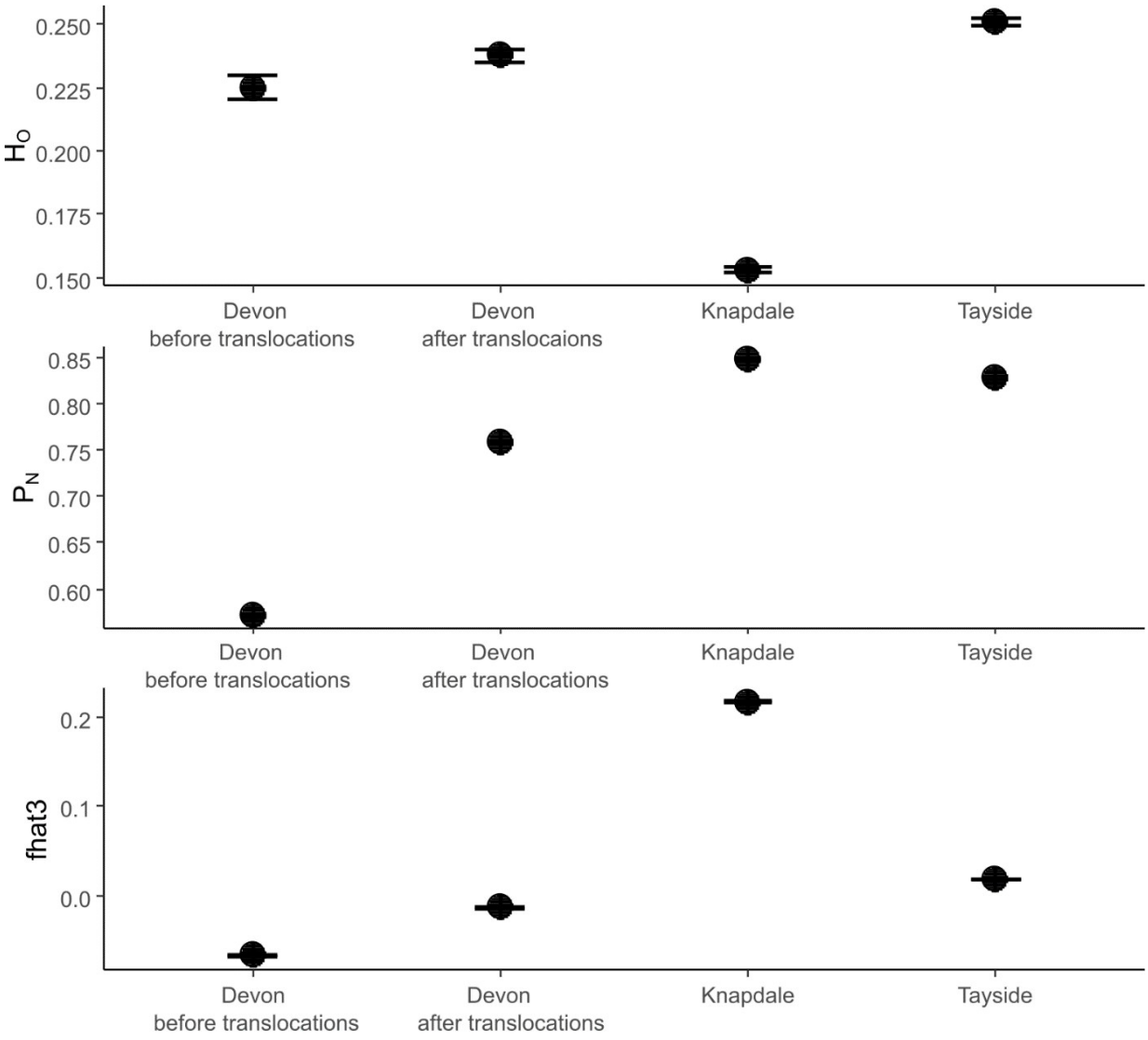

Figure 5. Graph of multiple genetic diversity statistics for the Devon population prior to translocation of individuals from Tayside, the Devon population after the addition of translocated individuals, a Knapdale reference population, and a Tayside reference population. H_O: observed heterozygosity, P_N: proportion of polymorphic markers, fhat3: average inbreeding coefficient. Bars represent the 95% confidence interval.

Secondly, we examined the pairwise relatedness between the Devon individuals to identify whether there are any close relatives in the population (Figure 6). The average relatedness across the sampled individuals in Devon (n = 5) outside the River Otter population was very low (r = - 5.464). However, there were four instances of close relations being identified: BEV745 and BEV754 (r = 0.294), BEV745 and BEV748 (r = 0.397), BEV746 and BEV747 (r = 0.355), and BEV748 and BEV754 (r = 0.435). All relatedness values are available in Appendix III Table 3.

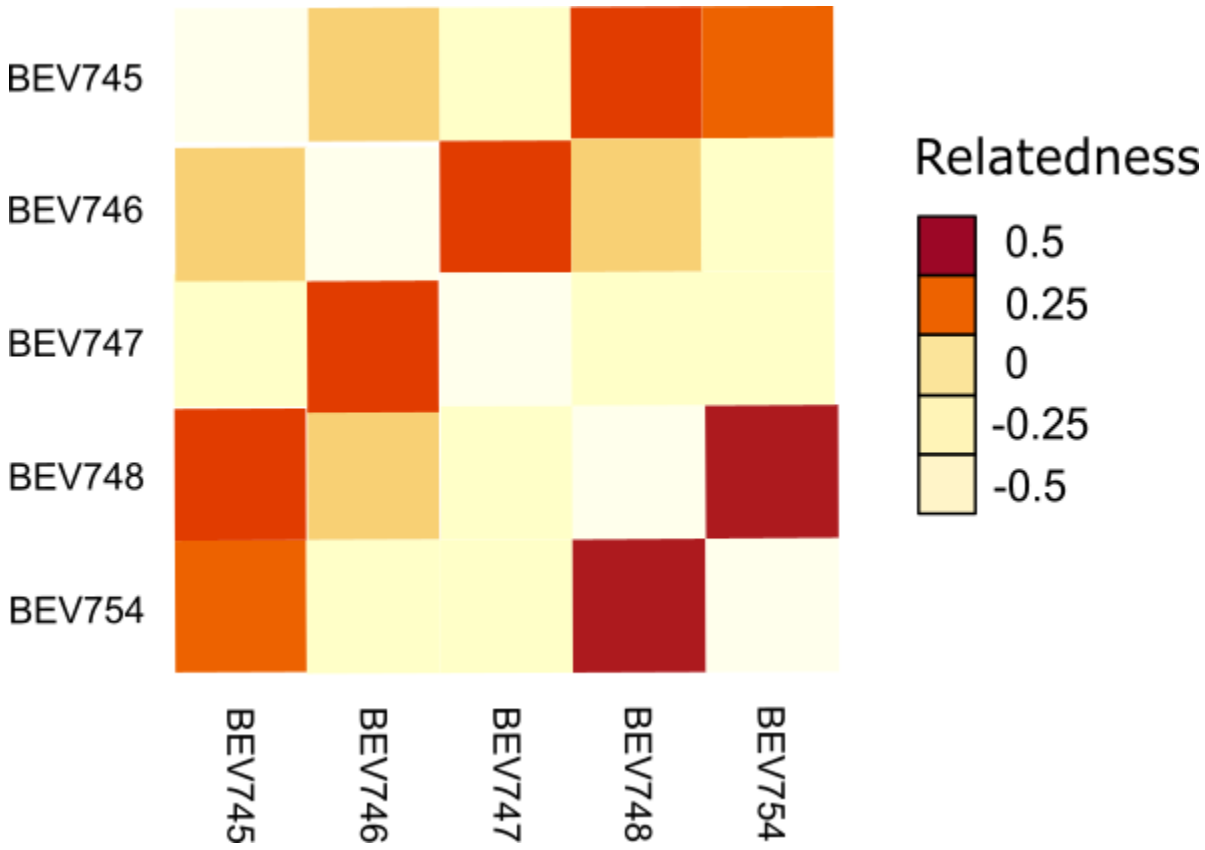

Figure 6. Heatmap of pairwise relatedness values between each pair of individuals. The darker the square the higher level of relatedness between those two individuals.

Thirdly, we compared all beavers in Devon to reference European populations to determine which European refugia they may have originated from (Table 5). All individuals examined here showed a high probability of originating from Bavaria, Germany.

Table 5. Proposed origin populations for the Devon beavers as determined by GeneClass2. P is the probability that an individual belongs to that population.

		GeneClass2 assignment			
Origin	**Individual**	**Assigned population 1**	**P**	**Assigned population 2**	**P**
Upcott, Devon	BEV745	Germany (Bavaria)	0.977	Germany (Baden-Württemberg)	0.370
Devon	BEV746	Germany (Bavaria)	0.751	Germany (Baden-Württemberg)	0.175
Boldventure, Devon	BEV747	Germany (Bavaria)	0.976	Germany (Baden-Württemberg)	0.375
Devon	BEV748	Germany (Bavaria)	0.976	Germany (Baden-Württemberg)	0.432
Devon	BEV754	Germany (Bavaria)	0.859	Germany (Baden-Württemberg)	0.232

Beavers captured from the wild in Kent and subsequently held in captivity

We received a total of two samples from individuals that were captured from the wild in Kent and subsequently held in captivity. At the time of receiving the blood samples they were being held in captivity with two additional beavers, one transferred from a German zoo and another wild caught in Tayside; we also received blood samples from both of these. While emphasis might be placed on the two individuals from the wild as these are from a larger free-living population, two individuals are insufficient to accurately inform the diversity of this population in Kent and thus we have not calculated genetic diversity statistics for this sample group.

Firstly, we examined the pairwise relatedness between the four individuals to determine whether any individuals are related to each other. The average relatedness across all individuals (n = 4) was very low (r = -205.024) which is unsurprising given that all four beavers were from different locations. No pairs of beavers showed any indication of being related (Figure 7) including the two beavers captured from the wild in Kent (BEV794 and BEV796, r = -0.389). All relatedness values are available in Appendix III Table 4.

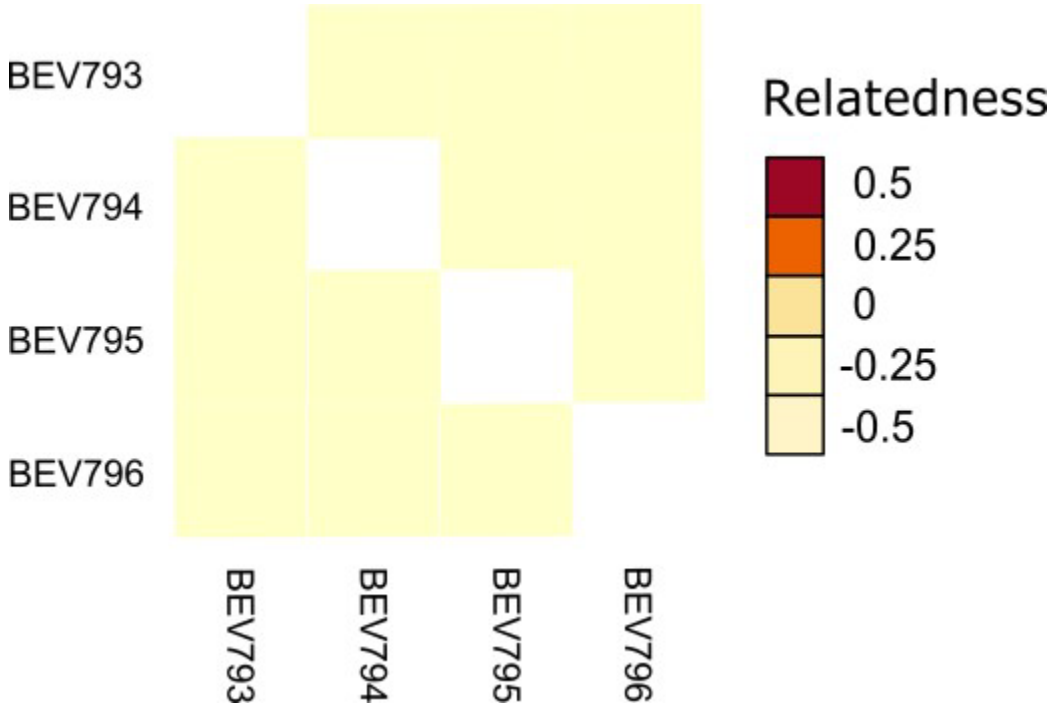

Figure 7. Heatmap of pairwise relatedness values between each pair of individuals. The darker the square the higher level of relatedness between those two individuals.

Secondly, we compared all beavers in Kent to the reference populations to determine which European refugia they may have originated from (Table 6). The two wild living beavers from Kent were determined to be most similar to the population in Tayside, Scotland although support for this was moderate (BEV794, P: 0.440) to low (BEV796, P: 0.061). The beaver from Wuppertal Zoo, Germany was determined to be most likely from Bavaria, Germany (with moderate support, P: 0.452) and the beaver from Tayside was confirmed to be most likely from Tayside, Scotland (with high support, P: 0.964).

The low probability assignments for some of these individuals could be due to admixture or because they originate from a currently unsampled beaver population. The STRUCTURE results (Figure 13) showed that the beaver from Eastry, Kent (BEV794) and the beaver from Tayside (BEV795) had signatures of admixture similar to the Belarus population. The beaver from Sandwich Bay, Kent (BEV796) showed a STRUCTURE signature more similar to the Southern German populations of Bavaria and Baden-Württemberg.

Table 6. Proposed origin populations for the Kent beavers as determined by GeneClass2. P is the probability that an individual belongs to that population.

		GeneClass2 assignment			
Origin	**Individual**	**Assigned population 1**	**P**	**Assigned population 2**	**P**
Wuppertal Zoo, Germany	BEV793	Germany (Bavaria)	0.452	Germany (Baden-Württemberg)	0.164
Eastry, Kent	BEV794	United Kingdom (Tayside)	0.440	Germany (Bavaria)	0.401
Tayside, Scotland	BEV795	United Kingdom (Tayside)	0.964	Germany (Baden-Württemberg)	0.080
Sandwich bay, Kent	BEV796	United Kingdom (Tayside)	0.061	Germany (Bavaria)	0.020

Analysis of the results and put into perspective in relation to Scottish and European populations

To understand how the English beaver populations compare to established Scottish and European populations we used both; a single mitochondrial DNA (mtDNA) locus and a larger ddRAD sequencing dataset.

mtDNA

Using mtDNA can give us an initial insight into the genetic diversity of a population. It can also be used for an initial assessment into which refugia population an individual may have originated from as the mtDNA locus used here has been shown to differ between beaver populations in Europe (Senn et al., 2014).

A total of five haplotypes were recovered across the 243 individuals we sampled (Figure 8). The most dominant haplotype observed across all populations was 'jf7' with haplotypes 'al1' and 'nh2' only being found in a small number of individuals. Haplotype counts for each population analysed are available in Appendix IV Table 1.

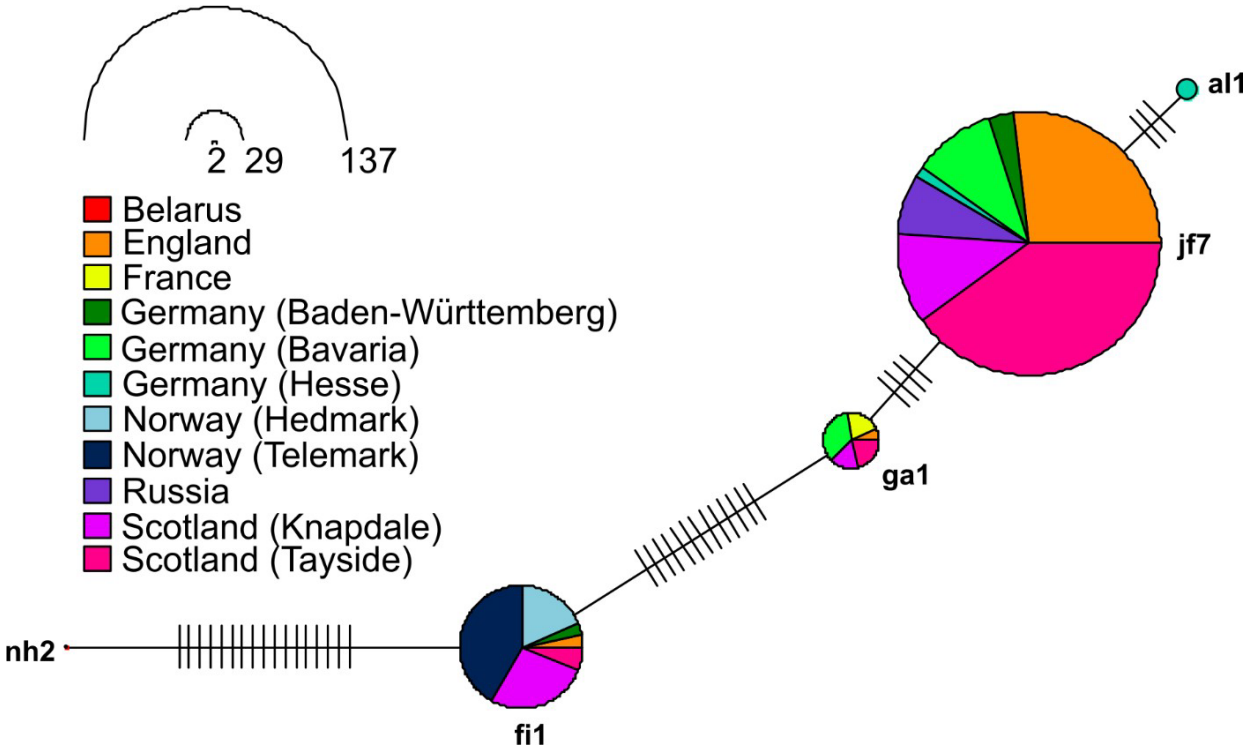

Figure 8. Haplotype network of the European and British beaver samples. Each population is represented by a unique colour, and the haplotype circles sizes are scaled by sample number.

Strikes between haplotype circles represent the number of mutational changes between each haplotype.

The reference populations in Norway, France, Germany (Hesse), Belarus and Russia correspond to the subspecies classified by Durka et al., (2005) (see Table 1 for details), and in our dataset each of these are associated with a single haplotype except for Germany (Hesse) (Figure 9). The Hesse population is slightly unusual, as it is a reintroduced population, with the founders thought to have been sourced from the Elbe river basin population (*Castor fiber* albicus). This association of a single haplotype to each fur-trade refugia suggests these populations are insular and may have reduced genetic diversity compared to ancestral populations. In previous work, these refugia were further split into two groups, Eastern (Poland, Lithuania, Russia, and Mongolia) and Western (Germany, Norway and France), thought to represent two distinct genetic lines that should not be mixed (Durka et al., 2005). This split has since been shown to be due to human hunting- associated reductions in populations (Horn et al., 2014), suggesting the apparent split is human- induced and that Eastern and Western populations do not necessarily have to be managed separately. Notwithstanding, care should be taken when discussing potential translocations of beavers from Russia as it has been shown that multiple refugia populations survived across this country (Durka et al., 2005). Indeed, the current study only examined one population from Russia that happened to harbour a characteristically Western haplotype (‘jf7’) and is therefore unlikely to be representative of all wild beaver populations within Russia.

Only populations that are known to result from reintroductions contained more than one mitochondrial haplotype. This was largely consistent with the documented origins of the population founders, which were often mixed. For example, the Bavarian population, documented to have been reintroduced using beavers of mixed origins (see Table 1) contained two of the five observed haplotypes. The Knapdale reintroduction is also known to result from beavers of mixed origin (although breeding between the two origin groups is yet to be seen) and contains three of the five haplotypes. An exception to this was the Baden-Württemberg population, thought to have originated from beavers from France which actually contains the ‘fi1’ and ‘jf7’ haplotypes found in Norway and Russia, respectively. However, there has been a rapid expansion of the beaver populations in Germany in recent decades (Halley et al., 2020) and it is likely that movement of individuals between the populations in Germany has occurred.

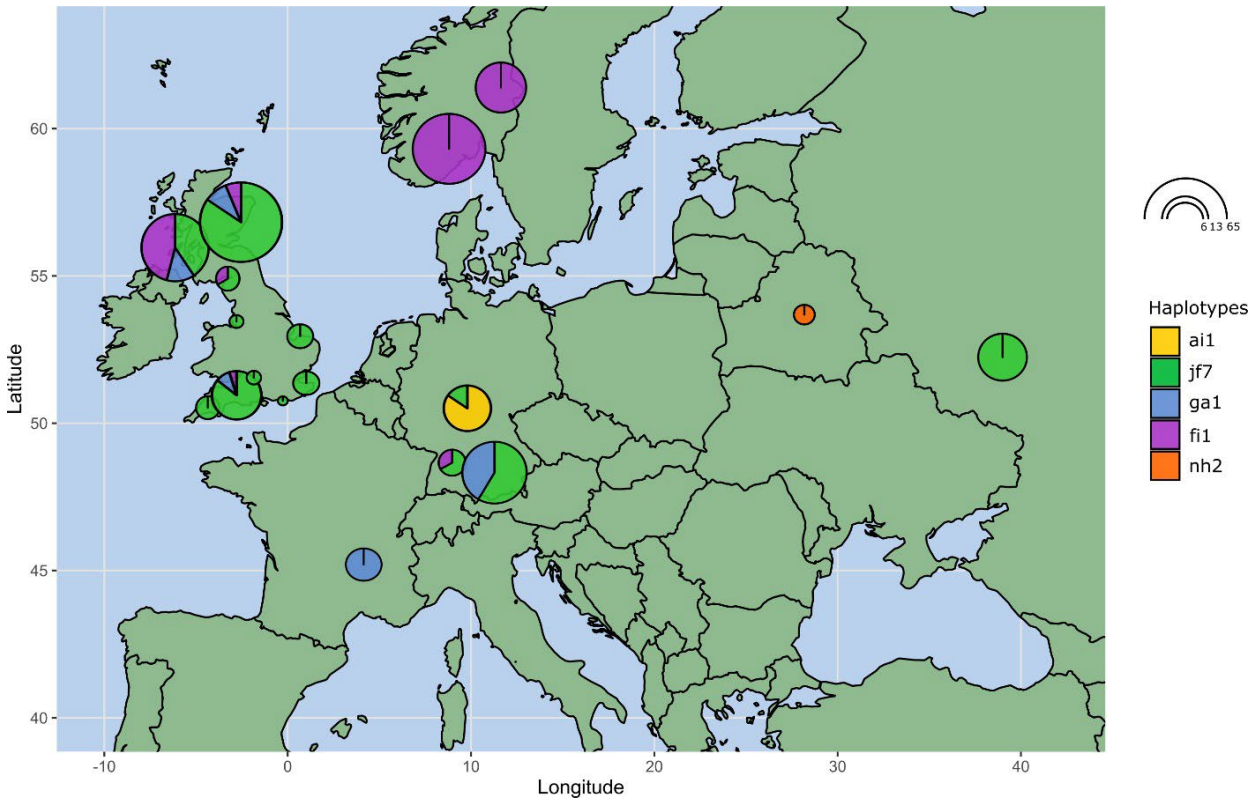

Figure 9. Map showing the proportion of haplotypes found per population across Europe based on data generated in this study. Pie charts are scaled by sample size.

Beaver populations in Great Britain exhibit three haplotypes but not all populations contain all three haplotypes (Figure 10). The established Scottish populations of Knapdale and Tayside do contain all three haplotypes. This reflects the translocated individuals from Norway (haplotype ‘fi1’), and individuals from Tayside of Bavarian origin (haplotypes ‘jf7’ and ‘ga1’).

The dominant haplotype of beavers in England is ‘jf7’ which has been previously reported for populations in Russia, reintroduced populations in Germany (Senn et al., 2014) and latterly, reintroduced populations in Scotland (unpublished). All individuals in Cheshire, Cornwall, Gloucestershire, Kent, Norfolk, and West Sussex are haplotype ‘jf7’ and are thus ultimately all descended from the same maternal line. In Devon, there are two individuals with the haplotype ‘ga1’, one of which was translocated from Tayside. There is also an individual in Devon with the haplotype ‘fi1’ which was also translocated from Tayside. In Cumbria, there is one individual translocated from Tayside with the haplotype ‘fi1’. Even after the translocation of individuals from Tayside the dominant haplotype in the English beaver populations is ‘jf7’.

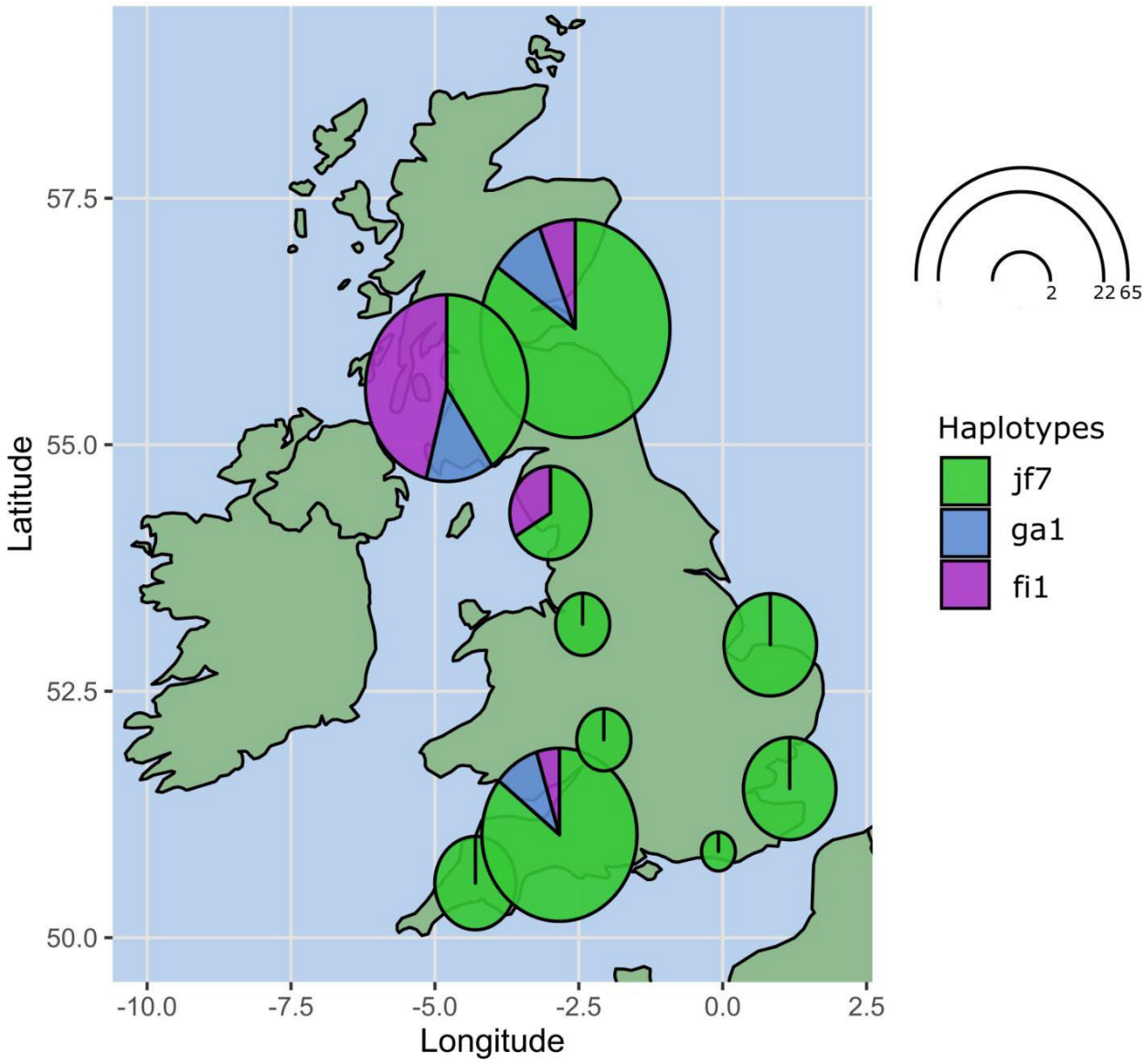

Figure 10. Map showing the proportion of haplotypes found per population across Great Britain. Pie charts are scaled by sample size.

ddRAD

Firstly, we compared the English beaver populations to multiple Scottish and European reference populations to examine the genetic diversity across all populations (Figure 11). If we consider the beavers in England as a single population, they have genetic diversity measures comparable to reference populations in Scotland and Europe. Genetic variability in England (HO = 0.242) is close to that in Tayside (HO = 0.251), and higher than all other populations examined. The proportion of polymorphic markers in beavers in England (PN = 0.836) is also close to those seen in the Tayside (PN = 0.829) and Knapdale (PN = 0.848) populations, and again is higher than all other populations examined. The amount of inbreeding in English beavers is moderate (fhat3 = 0.0427) and comparable to reference populations in Great Britain and Europe. However, it is important to be cautious when interpreting these results where the English beavers are considered to be a single population. Beavers in England are currently located across eight English counties; they are not a single breeding population like the reference populations to which they are being compared. The assumption made in this comparison would rely on either i) the beavers being released together into one river catchment or ii) continuous human-facilitated movement of breeding individuals between counties.

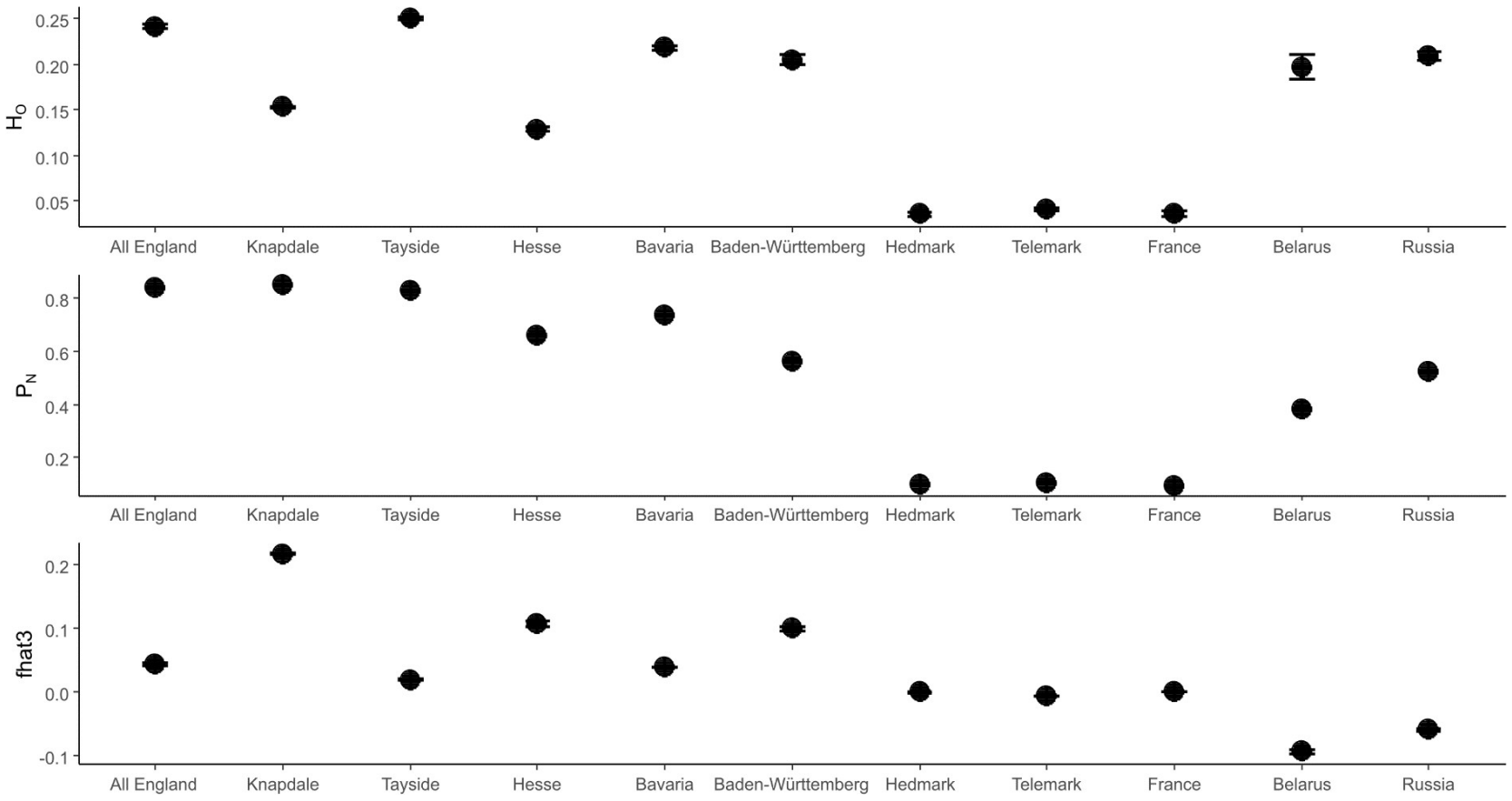

Figure 11. Graph of multiple genetic diversity statistics for the English beaver populations, Knapdale and Tayside Scottish reference populations, and all European reference populations. H_O: observed heterozygosity, P_N: proportion of polymorphic markers, fhat3: average inbreeding coefficient. Bars represent the 95% confidence interval.

Secondly, we examined broad patterns of genetic structure across all the English beavers and reference populations. A Principal Components Analysis (PCA) determined that the populations of Hesse, Germany; Hedmark, Norway; Telemark, Norway; and Knapdale, Scotland were vastly separated from the other populations examined (Figure 12A). Most of the other populations appear to have significant overlap but it was unclear to what extent. To determine the relationship between these populations a second PCA was run with the highly divergent populations noted above removed.

The revised PCA showed that the populations of France, Belarus and Russia are genetically distinct populations (Figure 12B). In contrast there is significant overlap between; the English populations; the Tayside, Scotland reference population; the Bavaria, Germany reference population; and the Baden-Württemberg, Germany reference population. This is in line with previously published reports on the Tayside and the River Otter beavers (Campbell-Palmer et al., 2020; Campbell- Palmer et al., 2015; McEwing et al., 2014), and the known recent translocations of beavers from Tayside to enclosures in England, which form the majority of the English samples in this analysis. The results reveal that the majority of beavers currently sampled in England originate from Tayside, Scotland and/or the Bavaria and Baden-Württemberg, Germany populations, where the Tayside, Scotland population is also largely comprised of individuals with Bavaria and Baden- Württemberg, Germany ancestry. However, the higher diversity found in the Tayside and subsequently English populations, compared to Southern Germany provides evidence that either

i) these populations may have ancestry from a yet unsampled population or ii) there is unsampled diversity within one (or more) of our reference populations.

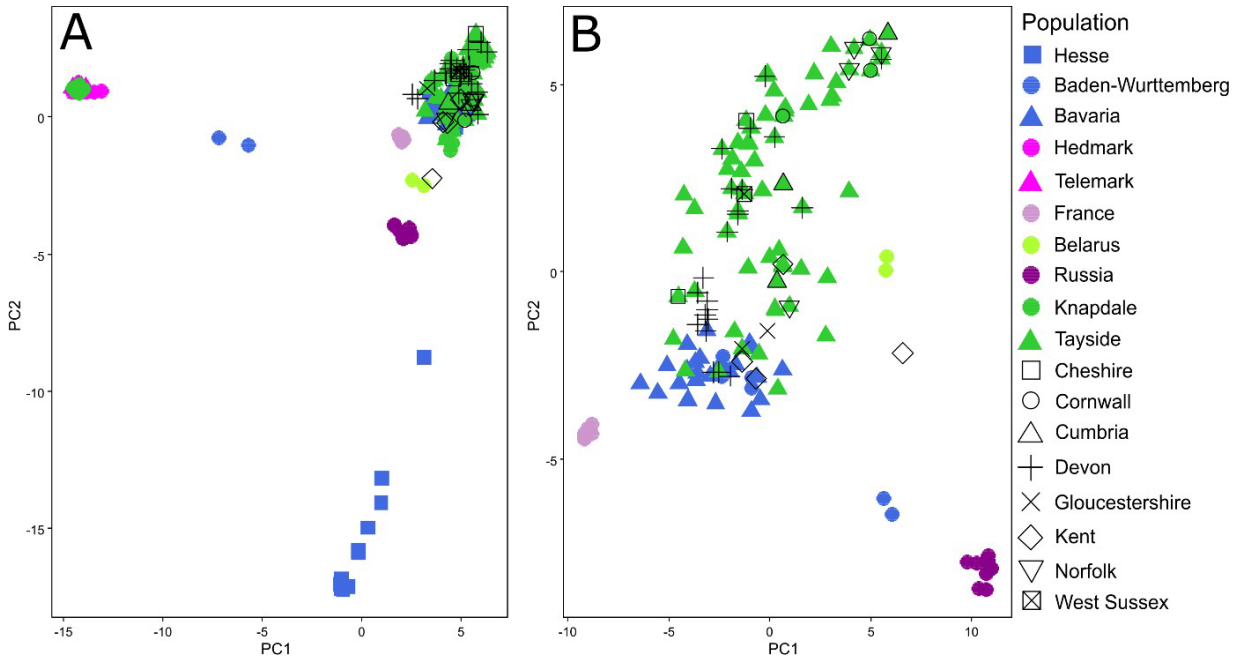

Figure 12. Principal components analysis of A) all English beaver populations and European reference populations and B) all populations excluding Hesse, Germany; Hedmark, Norway; Telemark, Norway; and Knapdale, Scotland. Each point represents one individual and colour denotes which country they belong to. Shape type is used to distinguish between populations in the same country.

In addition to the PCA analysis, we also undertook a STRUCTURE analysis to examine the population structuring between all English and reference samples. The STRUCTURE analysis showed a clear separation of the Hesse, Germany; Hedmark, Norway; Telemark, Norway; Belarus; Russia; and France populations (Figure 13) which agrees with the PCA analysis (Figure 12).

Individuals from the River Otter, Devon, and the individuals located in Devon prior to the recent translocations all had a high probability of originating from the Bavaria, Germany and the Baden-Württemberg, Germany cluster (Figure 13). This is in agreement with the population assignment analysis in Table 4 and Table 5, respectively, which also placed these beavers as most likely originating from Bavaria and Baden-Württemberg. Individuals that were translocated to Devon have a signature of originating from Tayside, which corroborates with the field data that states they were translocated from Tayside to Devon (pers. comm. Dr Róisín Campbell-Palmer).

All individuals translocated to England have a signature of originating from Tayside (K = 5, K = 6, K = 7; second, third and fourth panel, Figure 13) which is their known origin according to field data (pers. comm. Dr Róisín Campbell-Palmer). When only up to four genetic clusters are considered (K = 4; top panel, Figure 13) all translocated individuals are shown to have a similar admixture signature to Bavaria, Germany and Baden-Württemberg, Germany which, in turn, are shown to likely have a mixed ancestry of individuals from France, Belarus, Russia and Norway. The Tayside reference population was also shown to have the same admixture signature seen in previous genetic assessments that suggested individuals from Tayside were most likely to originate from Lithuania/Poland, Bavaria, Germany and Baden-Württemberg, Germany (Campbell-Palmer et al., 2020).

The admixture signature in Tayside beavers, and in the individuals translocated from Tayside to England, is particularly interesting. The STRUCTURE analysis suggests that there may be a signature of ancestry from the sampled Belarus population (although this population is only represented by two individuals in the current analysis) or from an unsampled population. This signature was also seen in the beavers from Kent that had low origin population assignment scores (Table 6). Further analysis that includes additional individuals from Belarus, and/or individuals from other European populations (e.g. Poland or Lithuania) would be required to provide clarity.

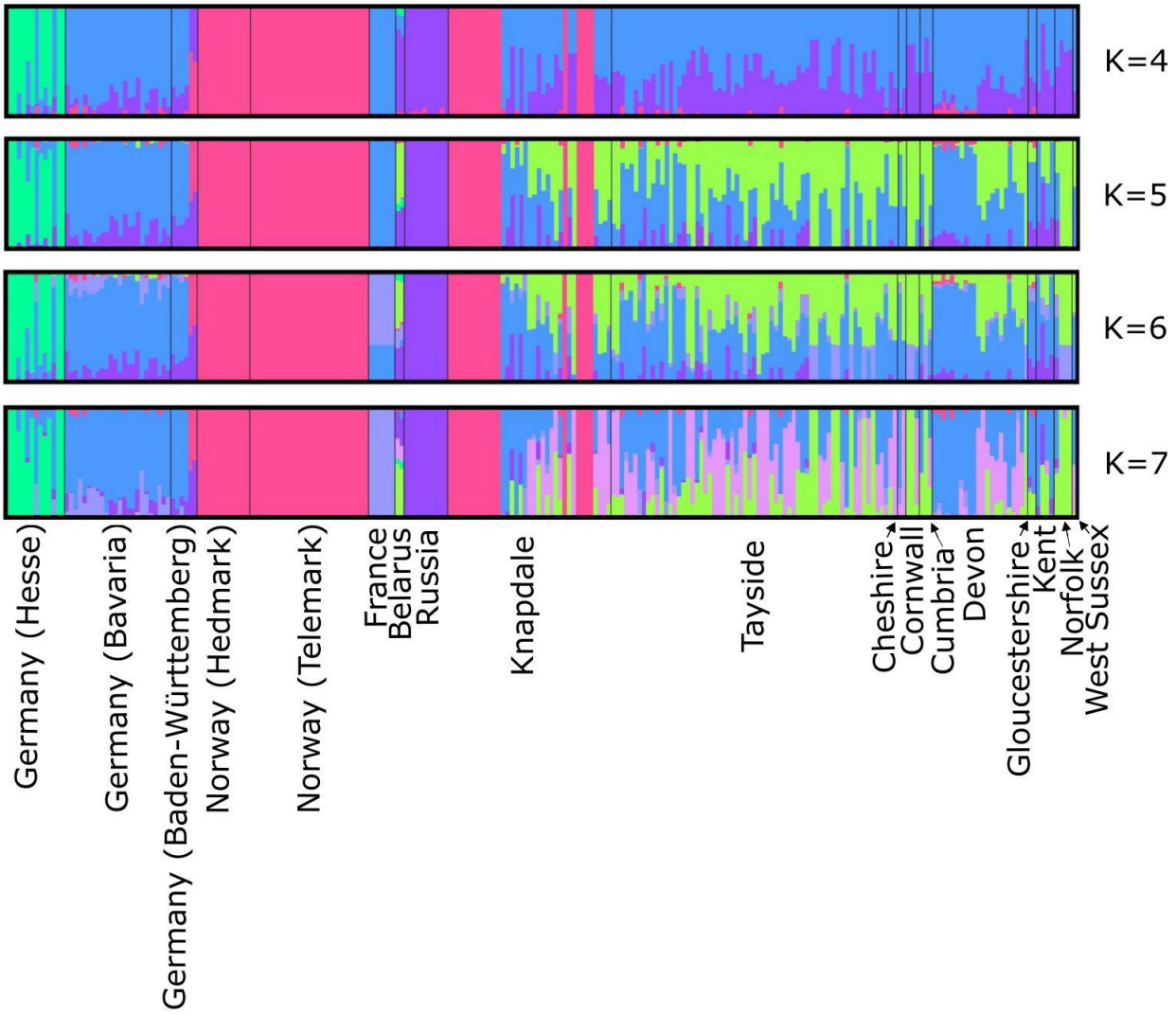

Figure 13. STRUCTURE plot for multiple iterations of clustering groups (K = 4, K = 5, K = 6, and K =7). Each vertical bar represents one individual where the proportion of colour indicates how likelythey are to belong to that genetic cluster. Individuals of the same colour are likely to have originated from the same population.

We combined the results from our data analysis to produce a map showing both known and hypothesised reintroduction routes of beavers across the European populations we examined (Figure 14). This map highlights the suggested movement of beavers and shows how certain populations (i.e. Bavaria, Germany) are of mixed origin. It also highlights that the beavers in England most likely have mixed origins from Tayside, Bavaria and Baden-Württemberg, Germany, and potentially a location in Eastern Europe.

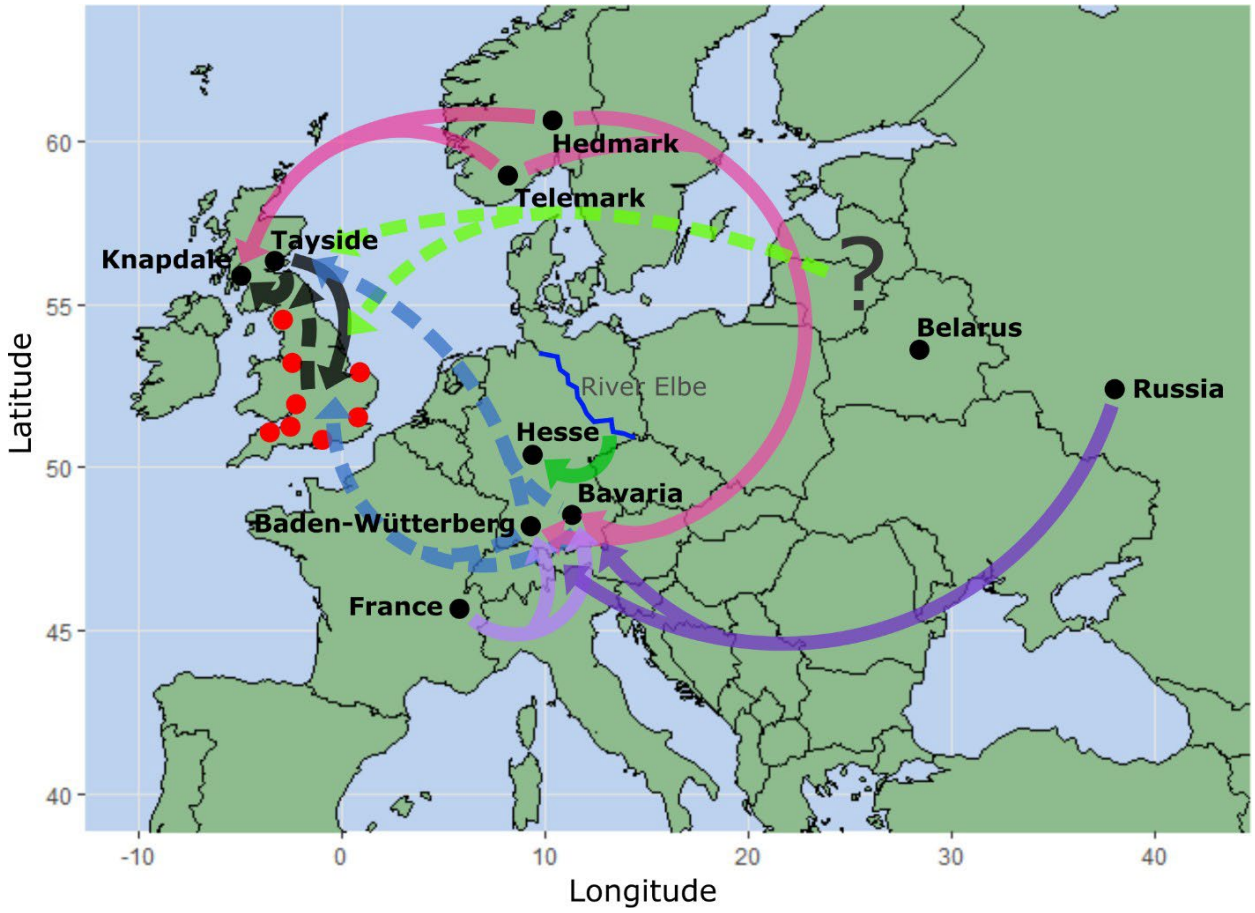

Figure 14. A map showing potential and known reintroduction routes of beavers across Europe inrelation to the founding of the British populations. Arrows show the direction of movement between populations where solid lines indicate known routes and dashed lines indicate hypothesised routes based on genetic data. Arrow colours represent the clades identified in the STUCTURE analysis. English locations are denoted by red circles.

Recommendations for a potential future reintroduction strategy

To maximise the success of a reintroduction of beavers into England, there are several key aspects to consider from a genetics perspective:

1. How much genetic diversity is there in the English beaver populations, and how does this compareto other established populations of beavers?
2. Is additional genetic diversity required for the maintenance of English beaver populations?
3. If additional genetic diversity is required, where should we source additional founder individuals?
4. How do we then maintain the genetic diversity in the reintroduced populations in England?

We will address each of these in turn regarding a potential future reintroduction strategy for beavers in England.

How much genetic diversity is there in the English beaver populations, and how does this compare to other established populations of beavers?

If beavers are to thrive in England in the long term, maximising genetic diversity is imperative. Genetic diversity is crucial for populations to adapt to changing circumstances and thus persist in the long term. Populations with low genetic diversity risk being wiped out by events such as disease or climate change, but those with more diversity stand a chance of adapting and surviving (Ellegren et al., 1993).

Genetic diversity across all the beavers in England is reasonable given the genetic history of Eurasian beavers and it is comparable to established populations in Scotland and mainland Europe. This suggests that beavers in England may be genetically viable. However, it is very important to note that these beavers were analysed as a metapopulation due to the limited number of available samples for beavers in England. Currently these beavers can't be considered as a single large population as in reality they represent multiple small groups (some free-living and others in licensed enclosures), isolated from one another across eight English counties. Additionally, the available samples for these populations were too limited to estimate the true extent of genetic diversity in free-living beavers in England.

Small, isolated populations will naturally experience loss of genetic diversity over time through genetic erosion (Méndez et al., 2014). Large population sizes and migration between populations is required to retain increased amounts of genetic diversity. It may be possible to maintain the current genetic diversity found within beavers in England, but this would require rapid population increases, extensive management and ongoing translocations between

populations and enclosures, which is yet to occur. If management as a metapopulation is not a feasible option then it is likely that additional individuals will need to be translocated into England.

Is additional genetic diversity required for the maintenance of English beaver populations?

Carefully selecting genetically diverse founders is even more important for species such as the Eurasian beaver, which have undergone recent drastic declines in genetic diversity, in the very recent past. This is evident in the very low genetic diversity seen in the French and Norwegian refugia populations which is likely due to historic population sizes falling as low as 30 and 60 individuals, respectively (Durka et al., 2005 and references therein), with no subsequent recolonisation events. Conversely, some Russian and Belarusian refugia are thought to have persisted with greater numbers of individuals and our analysis showed they had higher genetic diversity. However, that is not to say that the smaller amount of genetic diversity that persists in Norway and France is not extremely important. As the mitochondrial DNA attests, and in line with the taxonomic classifications, the five known refugia harbour different genetic variants. This is likely the reason we find that populations founded by multiple source populations (such as Bavaria) have higher genetic diversity than any of the individual source refugia.

Adding new individuals into a population not only has the potential to introduce new genetic variation, but it also immediately boosts population sizes and helps to reduce the chance of mating between related individuals. In addition to ensuring beavers are translocated from source populations that introduce new variation, care should also be taken to translocate a sufficient number of individuals. A limiting factor for this will be the number of individuals suitable for translocation that are available from various source populations, which may be low due to small source population sizes, logistical challenges, and financial cost (Berger-Tal et al., 2020). If additional beavers are translocated into England it must be decided whether they are translocated into one or multiple populations. As the number of individuals that can be translocated may be low, separating this small number of individuals into multiple populations may result in an insufficient number of founders to enable multiple successful reinforcements or reintroductions. However, if multiple populations were successfully established these could be used for future England-wide, or Britain-wide, translocations that would create a safety net for any populations that may be badly affected in the future due to issues such as conflict, environmental change, and disease (Akçakaya et al., 2007). Establishing multiple populations across England would allow for these to be managed as a metapopulation in the short-to-medium-term with a long-term goal of multiple, connected self-sufficient populations, with migration-based geneflow between them. This would, however, require ongoing human-assisted migration which has financial and animal welfare implications.

If additional genetic diversity is required, where should we source additional founder individuals?

When reintroducing a species for the first time (i.e., founding a new population), an appropriate starting point is to locate an extant population that is the most closely related to the population that went extinct. Information for beavers present in Britain before their extinction in the 16th century is sparse but morphological data gathered from museum skulls suggested that the extinct British beavers aligned most closely with the extant beavers from the Norwegian fur-trade refugia (Kitchener and Lynch, 2000). This was used as the rationale for the selection of the beavers translocated in the initial Knapdale beaver reintroduction. However, subsequent genetic analysis revealing the extremely low diversity present in the beavers of Norway factored into the later decision to augment the population with beavers with non-Norwegian origins. More recent genetic analysis has also suggested that beavers present in Britain before their extinction are (Marr et al., 2018).

The most genetically close to beavers from Western Europe as identified in Durka et al., (2005)

individuals of Norwegian and Bavarian origin that were translocated to Knapdale, Scotland as part of the Scottish beaver trial (2009-2014) were seen to acclimatise well (Dowse et al., 2020). Indeed, the total known fatalities among translocated individuals were 31% over 5 years for the Scottish Beaver Trial, and 24% over 3 years for the Scottish Beavers Reinforcement (Dowse et al., 2020). These values fall within the expected fatality ranges for translocations where first-year mortalities have been reported as 14% in Poland (Zurowski and Kasperczyk, 1988), 17% in Germany (Heidecke, 1986), and 36% in Netherlands (Nolet et al., 1997). The success of the reintroduction of beavers into Knapdale and Tayside showed that individuals translocated from European populations are capable of adapting to environments in Great Britain but not without loses.

Furthermore, the expansion and rapid increase of the Tayside population in Scotland (Campbell-Palmer et al., 2021) and the success of multiple reintroductions across Europe using beavers with mixed origins (Frosch et al., 2004; Halley et al., 2020, and see Table 1) suggests that there have been limited negative effects of the mixing of beavers from multiple European fur-trade refugia. Considering the similarities in climate and habitat found across Great Britain and the persistence of beavers with Norwegian and Central/Eastern European ancestry in Scotland, we advise that sourcing beavers with ancestry from any of the five fur-trade refugia included in this study are likely to be suitable for reintroduction into England. There are also likely long-term benefits to reintroductions that use beavers from multiple origins, associated with the increased genetic diversity that this would bring.

How do we then maintain the genetic diversity in the reintroduced populations in England?

If no new individuals are translocated into England it may be possible to combat the lack of connectivity between wild populations, and individuals in enclosures, by continuing to translocate individuals between all populations of beavers in England. Logistically the translocation of individuals within Great Britain is likely to be more feasible than continued

translocation of individuals from Europe into Great Britain, but this would require the cooperation of multiple stakeholders and coordination across multiple authorities. This strategy could also reduce the risk of importing new diseases and parasites into Great Britain although this would still require monitoring and veterinary screening of all individuals (Girling et al., 2019; Campbell-Palmer et al., 2021). However, our analyses have shown the English beaver samples originate largely from the same source population, limiting the pool of genetic diversity available even when moving beavers between different locations in Great Britain. More individuals could be translocated from Tayside especially with the potential for individuals to be translocated rather than lost to increasing lethal control licensing. The population in Knapdale, however, is still relatively small so the number that could be translocated from there would be limited. Even with an increase in individuals being translocated from Tayside (and potentially Knapdale) this may help to prevent the loss of genetic diversity but is unlikely to introduce new genetic variation. The translocation of any individuals, regardless of origin, should be considered carefully as there are animal welfare implications which could result in the loss of animals.

If new individuals are introduced into the English populations, as a source of additional genetic diversity, it is essential that this diversity is quickly assimilated to minimise any potential loses. The breeding success of founding members will dictate how much diversity is being introduced into a population but the rate of population expansion is ultimately the driver that affects how well the diversity is maintained (Allendorf and Luikart, 2007). As such, it is important that management strategies are developed to allow populations to expand rapidly.

Recommendations

Additional sampling and genetic analysis will ultimately be required to gain a more accurate understanding of the genetic diversity of beavers in England. Notwithstanding, it is our recommendation that additional beavers are translocated into the English populations to increase genetic diversity. Ideally these individuals should be from several European populations but can also include individuals from Tayside, and possibly Knapdale in the future. Suitability of the examined European populations are ranked based on available genetic diversity (PN), population estimates, and whether they are currently present in beaver populations in England (Table 7). Again, it should be noted that translocation of beavers from Russia will require additional investigations to determine their viability. These reinforcement efforts should be across all English populations and indeed Scottish populations; see beaver report recommendation (Dowse et al., 2020).

Table 7. Table outlining the available genetic diversity, population estimates* and current representation of the European populations in England. European populations are ranked for suitability for translocation to populations in England. Information presented here is based on the available reference data.

Population	Ranking	Genetic diversity (P_N)	Population estimates*	Current representation in England
Hesse, Germany	1	0.657	35,000	None
Baden-Württemberg, Germany	6	0.561		Medium
Bavaria, Germany	7	0.735		Medium
Voronezh, Russia‡	2	0.524	153,750	Low
Rhône, France	3	0.095	>14,000	Low
Telemark, Norway	4	0.105	>80,000	Low
Hedmark, Norway	5	0.096		Low
Neman, Belarus†	8	0.382	51,100	Medium

*as summarised in Halley et al., (2020)

‡requires further investigation of source population

†only based on 2 individuals

Conclusions

This study examined the genetic diversity of wild and translocated beavers in England and compared them to established Scottish and mainland European populations to provide genetic information for a potential reintroduction of beavers into England. All beavers sampled were genetically tested to examine how genetically diverse they are, to determine whether any close relatives were present within a given population, and to ascertain their source population. Examination of beavers translocated from Tayside showed they captured the known available genetic diversity within Tayside and, as such, have increased genetic diversity within beavers in England when considered as one metapopulation.

Despite the addition of translocated individuals into England, the populations there remain small and isolated. The majority of locations examined here are not wild-living populations but enclosures typically housing a single breeding pair. As such, they are at risk of genetic erosion, inbreeding, and potentially inbreeding depression. Although no official census estimates are available for the majority of the wild populations, the River Otter, Devon beaver trial has recorded an increase in known breeding pairs from two to seven, between 2015 and 2019 (Brazier et al., 2020) meaning even this actively managed population is small and had an extremely limited number of founders. We recommend that beavers in England require management as a metapopulation and/or wild populations of beavers need to be reinforced with additional translocated individuals. To maximise genetic variation, we recommend translocations from a variety of European populations, if feasible; additional translocations from the Tayside population in Scotland can also be considered. There may also be an opportunity for future translocations from the Knapdale population in Scotland, but this is unlikely to be possible in the near future.

Translocated beavers from Tayside have already provided additional genetic diversity to the English population overall and will be essential to reducing inbreeding in such small populations. Continued translocations and reinforcement measures will be essential in securing the long-term success of beaver reintroductions in England. Long-term genetic monitoring of these populations will be required to determine future risk of inbreeding depression, reductions in genetic variability, and to monitor population and range expansions. As with any translocation multiple factors have to be considered, of which genetics is one part, this report outlines some of the key challenges associated with this aspect but there are many others to consider and we advise that this report is used in conjunction with other guidance (e.g. on disease risk and animal welfare) as outlined in the IUCN translocation guidelines (IUCN, 2013).

Future work required:

1. A more representative sample set of wild-living beavers in England is required. In

this report we only had access to seven wild samples from two counties, Devon and Kent, however beavers have been observed in up to eight counties including Somerset and Herefordshire.

2. An increased sample set of European reference populations is required. We only had two individuals from the Belarus refugia population, and it has recently been suggested that additional fur-trade refugia populations may have persisted in Eastern Europe and in multiple locations in Belarus (Halley et al., 2020).
3. Future studies could benefit from the generation of more genetic data that covers a larger proportion of the genome, such as low coverage Whole Genome Sequencing (lcWGS). A greater volume of data would give more robust estimates of genetic diversity.
4. A population viability model investigating long-term population persistence given multiple alternative reintroduction scenarios, and using genetic data, would be helpful to plan an ongoing management strategy for beavers in England.

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Glossary

Admixture: mixing of two or more previously isolated groups.

Double digest restriction-site associated DNA (ddRAD): a technique that sequences many parts of an individual's genome which are then used to compare between individuals, and populations of individuals.

Genetic diversity: the amount of genetic differences between individuals.

Genetic structure: the pattern of sameness/differences within and between populations.

Haplotype: a specific DNA sequence that groups individuals together and is used to show relatedness.

Heterozygosity: where a genetic marker has two different DNA copies.

Inbreeding: mating of closely related individuals.

Inbreeding depression: the reduction in fitness and survival in the offspring of related individuals compared to the offspring of unrelated individuals.

Locus: a specific location on a gene or genetic region.

Mitochondrial DNA (mtDNA): maternally inherited DNA located in the mitochondria of cells.

Pairwise relatedness: a measure of how closely related two individuals are.

Polymorphic markers: genetic markers where variation is observed within a population.

Probabilities: statistical probability of how likely something is to be true.

Single nucleotide polymorphism (SNPs): a genetic site that varies among individuals.

Appendices

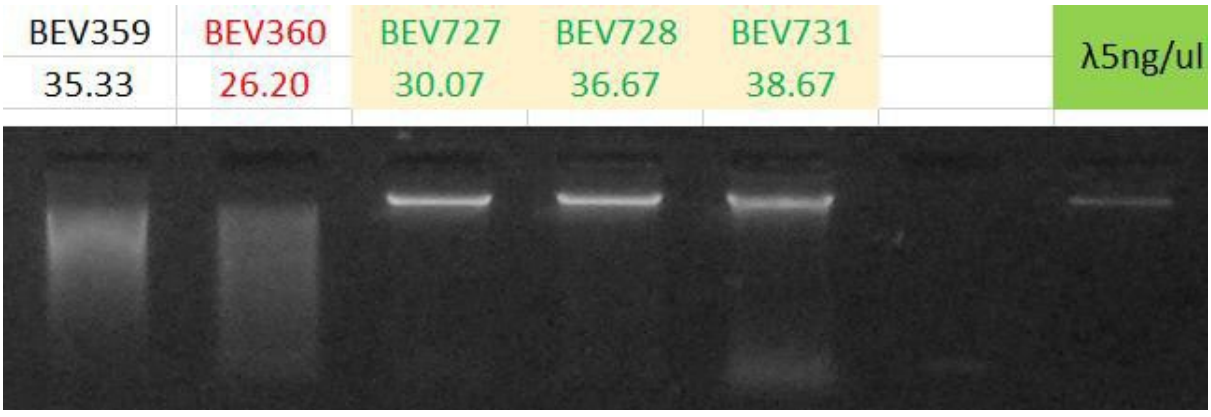
Appendix I: Full description of methods used for genetic data analysis

DNA extraction and quantification

DNA was extracted using Qiagen Blood and Tissue Kits and was successfully extracted from all 42 samples collected from beavers in England. The DNA concentration of all samples as measured by a fluorometer (Qubit® 2.0) are shown in Table 3. A total of two samples (BEV802 and BEV811, both from Devon) did not meet our minimum criteria of 5ng/µl concentration which is required for ddRAD sequencing. However, due to the limited number of samples available we did continue to process both samples by re-extraction and concentration of their DNA.

DNA quality checks

All samples collected from beavers in England except one (BEV802 from Devon) produced high molecular weight DNA as visualised via gel electrophoresis and recorded in Appendix I Table 1. Examples of high molecular weight DNA and degraded DNA are shown in Appendix I Figure 1.



Appendix I Figure 1. Example of the gel electrophoresis results conducted to visualize DNA quality of eachsample. The two samples on the left show degraded or fragmented DNA, the three samples from Devon (BEV727, BEV728 and BEV731) show high molecular weight DNA, and a 5ng/µl lambda standard (on the right) is included on each gel.

Appendix I Table 1. Summary DNA quality data for each sample obtained from beavers in England.

Sample ID	DNA concentration (ng/µl)	High molecular weight DNA	Number of reads produced during genomic ddRAD analysis	Location	Translocated to enclosure
BEV727	30.07	Yes	5721760	Devon	No
BEV728	36.67	Yes	8168429	Devon	No
BEV729	44.30	Yes	5604606	Devon	No
BEV730	43.80	Yes	8443879	Devon	No
BEV731	38.67	Yes	8572562	Devon	No
BEV745	29.5	Yes	13152165	Devon	No
BEV746	22	Yes	13080046	Devon	No
BEV747	35.5	Yes	11799163	Devon	No
BEV748	39.5	Yes	11315289	Devon	No
BEV754	30.6	Yes	9107542	Devon	No
BEV793	27.80	Yes	7939884	Kent	No
BEV794	40.40	Yes	4424430	Kent	No
BEV795	28.67	Yes	4261679	Kent	No
BEV796	24.20	Yes	6439469	Kent	No
BEV797	27.20	Yes	6968411	Gloucestershire	Yes
BEV801	15.40	Yes	3893486	Gloucestershire	Yes
BEV802	2.3	Not visible	3038	Devon	Yes
BEV803	18.20	Yes	5502589	Devon	Yes
BEV805	8.07	Yes	4317569	Devon	Yes
BEV806	9.27	Yes	4344804	Devon	Yes
BEV807	9.93	Yes	2891679	Cumbria	Yes
BEV808	13.80	Yes	4953009	Norfolk	Yes
BEV809	8.27	Yes	4160856	Norfolk	Yes
BEV810	8.80	Yes	3890193	Devon	Yes
BEV811	4.45	Yes	2777290	Devon	Yes
BEV812	10.80	Yes	4225012	Devon	Yes
BEV813	15.07	Yes	6575169	Devon	Yes
BEV814	12.27	Yes	4327810	Cornwall	Yes
BEV815	13.87	Yes	5338881	Cumbria	Yes
BEV816	9.80	Yes	7758414	Devon	Yes
BEV817	11.20	Yes	9557157	Devon	Yes
BEV818	7.53	Yes	3231129	Cornwall	Yes
BEV819	9.60	Yes	4485036	Devon	Yes
BEV820	14.87	Yes	8651581	Devon	Yes
BEV821	20.13	Yes	3962907	Norfolk	Yes
BEV822	15.73	Yes	9713067	Cornwall	Yes
BEV823	16.47	Yes	4095617	Norfolk	Yes
BEV824	24.80	Yes	8651267	Devon	Yes
BEV825	10.13	Yes	7779945	West Sussex	Yes
BEV826	14.93	Yes	4547686	Cheshire	Yes
BEV827	16.93	Yes	13334773	Cumbria	Yes
BEV828	36.2	Yes	8366416	Cheshire	Yes

mtDNA sequencing

Samples were sequenced at 405 bp of the mtDNA control region locus using a Sanger sequencing approach. Sequence quality was checked before alignment was performed in Geneious Prime version 2021.1.1. Sequences were then compared to previously published data (Durka et al., 2005; Senn et al., 2014) to determine the haplotype identity of each individual.

ddRAD library

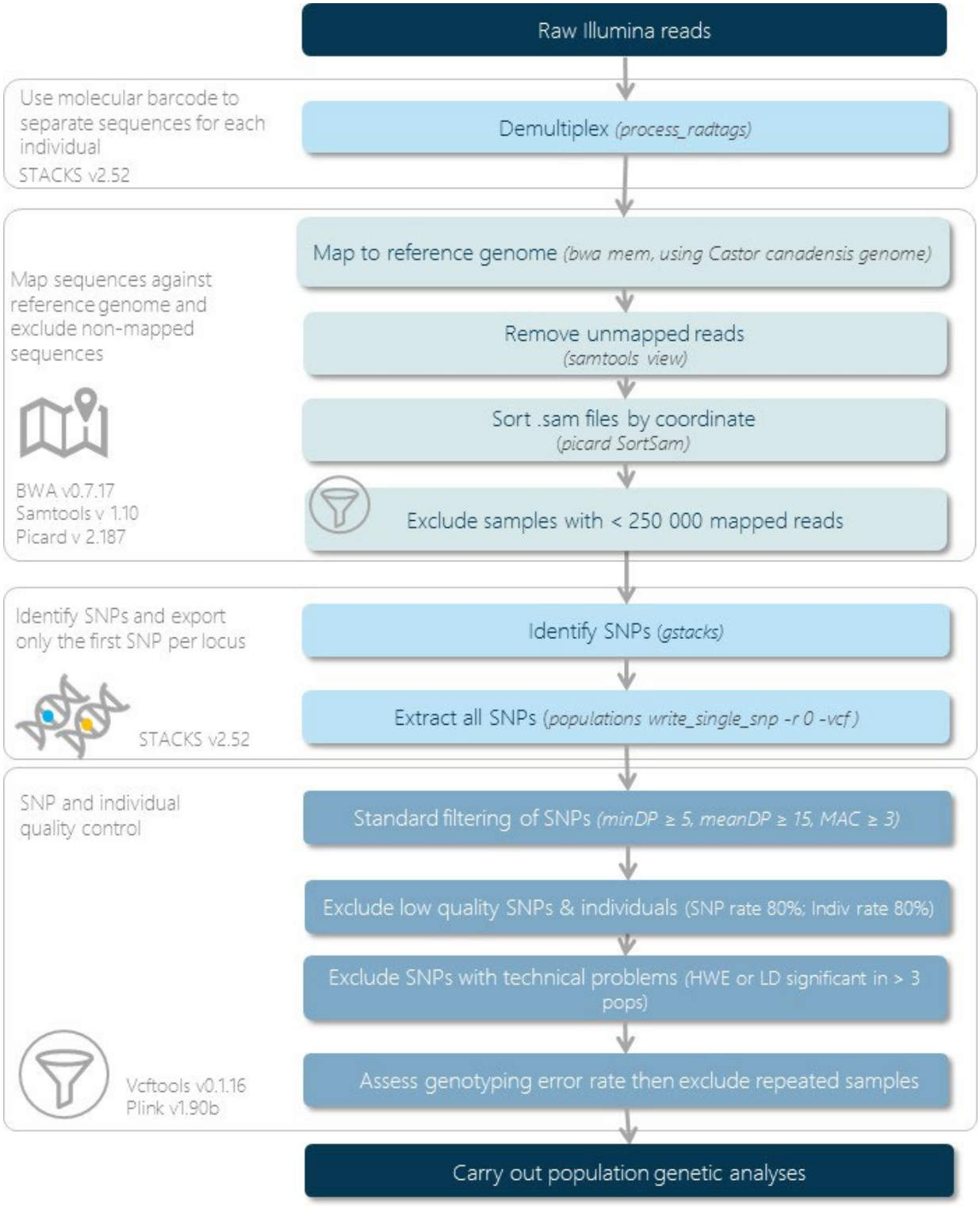
All 42 samples from England were prepared for next-generation sequencing via the Illumina Hi-seq platform. The 215 reference samples were also prepared using the same method. Up to 96 individually barcoded samples were prepared in each library, with the reference and English samples being run across three ddRAD libraries. The method used was based on that outlined by Peterson et al., (2012) with slight adjustments (Bourgeois et al., 2018). This sequencing method produces thousands of 130bp sequences per sample that can then be used to identify the variable regions or Single Nucleotide Polymorphisms (SNPs) across the genome. The number of sequences (or reads) produced for each sample can differ widely but we used a cut-off of a minimum 250,000 reads to exclude samples from downstream analysis as these are unlikely to produce robust genotypes. Read numbers for each sample collected from beavers in England are included in Appendix I Table 1. Using our cut-off criteria only one sample from England (BEV802) and 12 reference samples were excluded from the next phase of the analysis.

Bioinformatics

During ddRAD library preparation each sample was individually indexed before sequencing on an Illumina Hi-Seq platform. After sequencing, the millions of sequencing reads needed to be correctly allocated to each individual in a process known as demultiplexing. The sequencing data were demultiplexed and run through our standard bioinformatic pipeline as outlined in Appendix I Figure 2. This included the use of the North American Beaver[5] (*Castor canadensis*) genome to identify variable regions or SNPs. After completion, quality checks were performed using the positive controls, and both internal plate repeats and between plate repeats were used to check for genotyping accuracy. After all SNP and individual quality controls were completed,

[5] The North American beaver is the sister species of the Eurasian beaver and already has a published whole genome sequence (Lok et al., 2017). Use of whole genomes can improve the reliability of the final SNP panel (Galla et al., 2019).

repeated individuals were compared and the sample with the highest genotyping coverage was retained. A total of 1938 SNPs were retained for downstream analysis.



Appendix I Figure 2. Details of SNP calling and filtering pipeline for the ddRAD data used in this project.

Genetic analyses – mtDNA

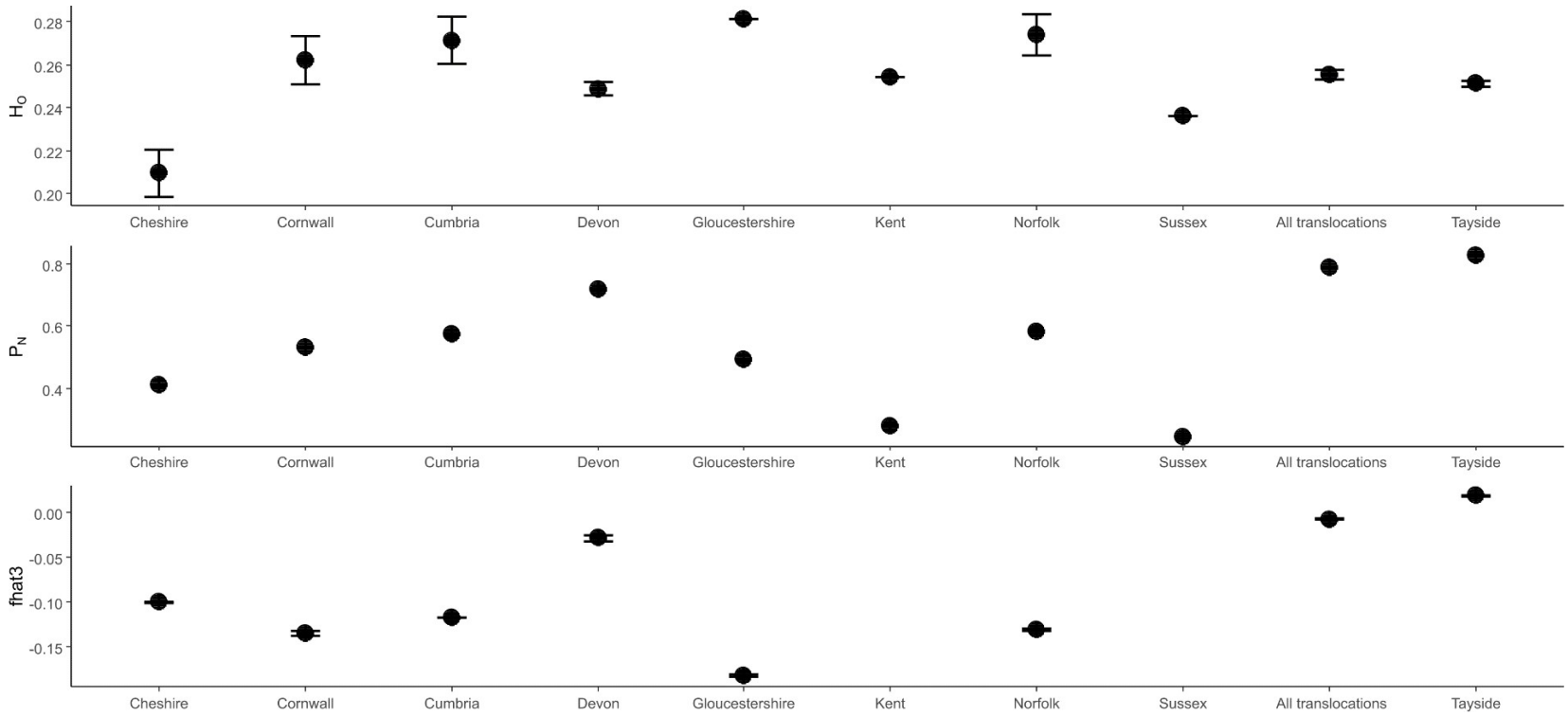
Haplotypes for all the English beavers were determined by comparison of the mtDNA sequences with previously generated data. We then used this to examine the distribution of haplotypes for beavers across Great Britain and Europe. As certain haplotypes have been associated with specific refugia this provides us with an initial assessment of which refugia population individuals may have originated from. A haplotype network of all individuals was constructed in R using the ‘pegas’ package. Pie charts of the haplotype composition of each population were plotted on a map in R using the ‘scatterpie’ and ‘rworldmap’ packages.

Genetic analyses - ddRAD

We examined multiple measures of genetic diversity for all English beaver populations to determine how diverse each enclosure and wild-living population is and how translocation has affected the overall diversity in England. We also determined these diversity values for Scottish and European populations as reference populations for the English beavers. Genetic diversity statistics of observed heterozygosity (HO), proportion of polymorphic markers (PN), and average inbreeding coefficient (fhat3) were calculated using PLINK v.1.90b (Purcell et al., 2007).

Pairwise relatedness was examined between all the individuals that were translocated from Tayside to the English populations as this allows us to test how closely related the individuals are. This is important to determine when working with small and/or potentially inbred populations to reduce the potential of breeding between close relatives once individuals are translocated. Relatedness was also examined between individuals in the River Otter, Devon; Devon (2016); and Kent. Understanding relatedness of individuals in these populations is important for determining how inbred these populations are or could become. Pairwise relatedness was calculated in CoAncestry using the Wang estimator (Wang, 2011).

While mtDNA analysis can give us an indication of which refugia population each beaver is likely to have originated from we can supplement this analysis with our larger ddRAD dataset. We used GeneClass2 (Piry et al., 2004) population assignment analysis to determine the most likely origin populations for each of the English beavers. Genetic structure was also analysed using a Principal Component Analysis (PCA) undertaken in R using the package ‘adegenet’ and using STRUCTURE v.2.3.4 (Pritchard et al., 2000).



Appendix II Figure 1. Graph of multiple genetic diversity statistics for each translocated group, all translocated individuals, and a Tayside reference population. H_O: observed heterozygosity, P_N: proportion of polymorphic markers, fhat3: average inbreeding coefficient. Bars represent the 95% confidence interval.

Appendix III Table 1. Table showing pairwise relatedness between all translocated individuals. Individuals in red (R) have shown some level of relatedness.

		BEV826	BEV828	BEV814	BEV818	BEV822	BEV807	BEV815	BEV827	BEV803	BEV805	BEV806	BEV810	BEV811	BEV812	BEV813
Cheshire	BEV826	NA														
	BEV828	-0.010	NA													
Cornwall	BEV814	-0.143	-0.110	NA												
	BEV818	-0.126	-0.155	0.397(R)	NA											
	BEV822	-0.052	0.087	-0.014	-0.040	NA										
Cumbria	BEV807	0.005	-0.174	-0.086	-0.071	-0.173	NA									
	BEV815	-0.154	-0.111	0.450(R)	0.438(R)	-0.042	-0.105	NA								
	BEV827	-0.054	0.001	0.018	-0.054	-0.029	0.019	-0.080	NA							
Devon	BEV803	0.076	0.142(R)	-0.007	-0.061	0.059	-0.018	-0.053	0.090	NA						
	BEV805	0.054	0.186(R)	0.037	-0.036	0.163(R)	-0.014	0.002	0.085	0.492(R)	NA					
	BEV806	-0.103	-0.128	-0.085	0.004	-0.066	-0.015	-0.028	-0.037	-0.007	0.002	NA				
	BEV810	-0.199	-0.190	0.425(R)	0.364(R)	-0.100	-0.146	0.461	-0.117	-0.040	-0.050	-0.141	NA			
	BEV811	0.074	0.031	-0.047	-0.074	0.031	-0.022	-0.090	-0.023	0.215(R)	0.184(R)	-0.115	-0.050	NA		
	BEV812	0.225(R)	0.088	0.023	0.030	0.070	0.051	-0.031	0.017	0.130(R)	0.173(R)	0.014	-0.080	0.438(R)	NA	
	BEV813	0.117(R)	0.070	0.052	0.011	0.467(R)	-0.059	0.020	0.030	0.088(R)	0.161(R)	0.015	-0.054	0.095	0.144(R)	NA
	BEV816	0.218(R)	0.024	0.022	-0.032	0.043	0.021	-0.017	0.026	0.172(R)	0.137 (R)	-0.070	-0.048	0.478(R)	0.487(R)	0.181(R)
	BEV817	0.056	-0.132	-0.265	-0.283	-0.223	-0.105	-0.252	-0.202	-0.108	-0.162	-0.185	-0.318	-0.093	0.047	-0.116
	BEV819	0.215(R)	0.056	-0.123	-0.099	-0.014	-0.047	-0.142	-0.098	0.193(R)	0.102	-0.089	-0.155	0.398(R)	0.500(R)	0.047
	BEV820	0.059	0.228(R)	-0.102	-0.107	0.108	-0.056	-0.143	-0.007	0.269(R)	0.470(R)	-0.103	-0.118	0.138(R)	0.122	0.064
	BEV824	0.144	0.021	-0.119	-0.098	0.012	-0.010	-0.114	0.047	0.007	0.013	-0.121	-0.156	-0.020	0.036	0.066
Gloucestershire	BEV797	0.027	-0.136	-0.084	-0.129	-0.136	0.035	-0.127	-0.084	-0.111	-0.065	-0.003	-0.132	-0.014	0.039	-0.036
	BEV801	0.091	-0.145	-0.108	-0.144	-0.231	0.006	-0.170	-0.115	-0.035	-0.043	-0.153	-0.216	0.085	0.095	-0.118
Kent	BEV795	-0.146	-0.248	-0.172	-0.070	-0.130	0.429(R)	-0.119	-0.070	-0.054	-0.078	0.459(R)	-0.197	-0.109	-0.013	-0.057
Norfolk	BEV808	0.034	-0.224	-0.007	-0.089	-0.201	0.210	-0.115	-0.076	-0.162	-0.111	-0.194	-0.102	-0.038	0.104	-0.066
	BEV809	-0.153	-0.049	0.388(R)	0.427(R)	0.022	-0.121	0.475(R)	0.004	-0.015	-0.033	-0.093	0.368(R)	-0.081	0.026	0.046
	BEV821	-0.006	0.031	0.369(R)	0.356(R)	0.043	-0.083	0.457(R)	-0.065	0.010	0.076	-0.010	0.400(R)	-0.010	0.033	0.058
	BEV823	-0.116	-0.115	0.430(R)	0.469(R)	-0.074	-0.053	0.421(R)	0.014	0.031	-0.002	-0.029	0.413(R)	0.003	0.009	0.025
West Sussex	BEV825	0.073	-0.057	-0.069	-0.139	0.044	-0.050	-0.103	0.015	0.054	0.074	-0.145	-0.155	0.073	0.082	0.074

		BEV816	BEV817	BEV819	BEV820	BEV824	BEV797	BEV801	BEV795	BEV808	BEV809	BEV821	BEV823	BEV825
Cheshire	BEV826													
	BEV828													
Cornwall	BEV814													
	BEV818													
	BEV822													
Cumbria	BEV807													
	BEV815													
	BEV827													
Devon	BEV803													
	BEV805													
	BEV806													
	BEV810													
	BEV811													
	BEV812													
	BEV813													
	BEV816	NA												
	BEV817	-0.028	NA											
	BEV819	0.461(R)	-0.073	NA										
	BEV820	0.113	-0.063	0.064	NA									
	BEV824	0.051	-0.142	0.021	-0.047	NA								
Gloucester shire	BEV797	-0.009	-0.044	-0.020	-0.108	-0.016	NA							
	BEV801	0.088	0.162(R)	-0.020	-0.039	-0.073	-0.006	NA						
Kent	BEV795	-0.065	-0.215	-0.077	-0.118	-0.115	0.034	-0.127	NA					
Norfolk	BEV808	0.000	-0.164	-0.065	-0.155	-0.156	0.045	0.055	-0.079	NA				
	BEV809	-0.010	-0.221	-0.095	-0.155	0.018	-0.139	-0.130	-0.191	-0.075	NA			
	BEV821	0.025	-0.184	0.030	-0.058	-0.091	-0.049	-0.080	-0.124	-0.009	0.423(R)	NA		
	BEV823	0.029	-0.206	-0.065	-0.103	-0.065	-0.138	-0.182	-0.115	-0.103	0.359(R)	0.308(R)	NA	
West Sussex	BEV825	0.114	-0.075	0.042	0.033	0.248(R)	0.009	-0.014	-0.093	-0.002	-0.068	-0.059	-0.134	NA

Appendix III Table 2. Table showing pairwise relatedness between all River Otter individuals. Individuals in red (R) have shown some level ofrelatedness.

	BEV727	BEV728	BEV729	BEV730	BEV731
BEV727	NA				
BEV728	0.542(R)	NA			
BEV729	0.467(R)	0.481(R)	NA		
BEV730	0.560(R)	0.495(R)	0.484(R)	NA	
BEV731	0.365(R)	0.541(R)	0.606(R)	0.263(R)	NA

Appendix III Table 3. Table showing pairwise relatedness between all Devon individuals. Individuals in red (R) have shown some level of relatedness.

	BEV745	BEV746	BEV747	BEV748	BEV754
BEV745	NA				
BEV746	0.053	NA			
BEV747	-64.463	0.355(R)	NA		
BEV748	0.397(R)	0.075	-0.012	NA	
BEV754	0.294(R)	-18.939	-0.154	0.435(R)	NA

Appendix III Table 4. Table showing pairwise relatedness between all Kent individuals. Individuals in red have shown some level of relatedness.

	BEV793	BEV794	BEV795	BEV796
BEV793	NA			
BEV794	-530.417	NA		
BEV795	-217.899	-338.510	NA	
BEV796	-142.655	-0.3888	-0.2713	NA

Appendix III Table 5. Table documenting field observations of suspected relations and corresponding pairwise relatedness analysis.

Population	Individual	Population	Individual	Field observed relationships	Genetics agrees?	Relatedness score
Devon	BEV806	Kent	BEV795	BEV806 mother/older sibling to BEV795	Yes	0.459
Devon	BEV805	Devon	BEV803	BEV805 mother to BEV803	Yes	0.492
Devon	BEV811	Devon	BEV812	Siblings	Yes	0.438
Devon	BEV811	Devon	BEV819	Siblings	Yes	0.398
Devon	BEV812	Devon	BEV819	Siblings	Yes	0.500
Devon	BEV816	Devon	BEV811	BEV816 father to BEV811	Yes	0.478
Devon	BEV816	Devon	BEV812	BEV816 father to BEV812	Yes	0.487
Devon	BEV816	Devon	BEV819	BEV816 father to BEV819	Yes	0.461
Cornwall	BEV814	Cornwall	BEV818	Siblings	Yes	0.397
Devon	BEV817	Cumbria	BEV815	Possible siblings	No	-0.252
Devon	BEV817	Cornwall	BEV814	Siblings	No	-0.265
Devon	BEV817	Cornwall	BEV818	Siblings	No	-0.283
Cumbria	BEV815	Cornwall	BEV814	Siblings	Yes	0.450
Cumbria	BEV815	Cornwall	BEV818	Siblings	Yes	0.438
Norfolk	BEV821	Norfolk	BEV823	Relation	Yes	0.308
Norfolk	BEV821	Devon	BEV817	Sibling	No	-0.184
Norfolk	BEV821	Cumbria	BEV815	Sibling	Yes	0.457
Norfolk	BEV821	Cornwall	BEV814	Sibling	Yes	0.369
Norfolk	BEV821	Cornwall	BEV818	Sibling	Yes	0.358
Norfolk	BEV823	Devon	BEV817	Sibling	No	-0.206
Norfolk	BEV823	Cumbria	BEV815	Sibling	Yes	0.421
Norfolk	BEV823	Cornwall	BEV814	Sibling	Yes	0.430
Norfolk	BEV823	Cornwall	BEV818	Sibling	Yes	0.469

Appendix IV: Haplotype data

Appendix IV Table 1. Table of the haplotype counts for the reference European populations and theEnglish groups.

		Haplotype Counts				
Population	**No. of Individuals**	**al1**	**jf7**	**ga1**	**fi1**	**nh2**
Reference Populations						
Germany (Hesse)	13	11	2	0	0	0
Germany (Bavaria)	24	0	14	10	0	0
Germany (Baden-Württemberg)	6	0	4	0	2	0
Norway (Hedmark)	12	0	0	0	12	0
Norway (Telemark)	27	0	0	0	27	0
France	6	0	0	6	0	0
Belarus	2	0	0	0	0	2
Russia	10	0	10	0	0	0
Knapdale, Scotland	37	0	15	5	17	0
Tayside, Scotland	65	0	55	6	4	0
English Locations						
Cheshire	2	0	2	0	0	0
Cornwall	3	0	2	0	0	0
Cumbria	3	0	2	0	1	0
Devon	22	0	19	2	1	0
Gloucestershire	2	0	2	0	0	0
Kent	4	0	4	0	0	0
Norfolk	4	0	4	0	0	0
West Sussex	1	0	1	0	0	0

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