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## HEALTH AND GENETIC SCREENING REPORT FOR WILD BEAVERS ON THE RIVER OTTER, DEVON.

CAMPBELL-PALMER, GIRLING, SENN & PIZZI, APRIL 2015.

*Five wild trapped beavers of unknown origin and living on the River Otter, Devon, were health screened for a range of parasites and disease of concern. Additionally samples were collected for genetic confirmation of beaver species, genetic diversity and degree of relatedness. All individuals were in good to very good body condition and appear to be adapting and surviving well in an English landscape with no obvious health and welfare concerns. They were confirmed as Eurasian beavers, most likely part of one extended family with closely related pairings.*

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## Background

Two families of breeding beavers were reported on the River Otter, Devon, in February 2014. After a successful public campaign to see them remain in place, Natural England granted a 5 year licence to release these beavers. A condition of this licence was that the animals were healthy and free of any diseases and parasites of concern. Though beavers are not especially recorded as being major reservoir of disease, as rodents they can harbour pathogens of health concern. *Echinococcus multilocularis* (henceforth abbreviated to EM) has been recorded in an imported beaver in an English captive collection. The origin and health status of the River Otter animals are unknown and may be of concern, particularly if they carry non-native parasites such as EM or diseases (such as Tularemia, *Francisella tularensis*). There is also a need to clarify that the North American species (*Castor canadensis*) has not been released and that any trapped beavers are Eurasian (*C. fiber*). Further genetic analysis, including degree of relatedness and genetic diversity, would inform any further animal releases. An additional consideration of any health screening was to assess the adaptability of beavers to survive in an English landscape after an absence of over 400 years.

### AIMS

- To determine these beavers were free from the non-native parasite EM and non-native disease Tularemia.
- To determine these beavers were free from diseases and parasites of concern to human and livestock health, such as bovine TB.
- To determine the general health status and body condition of all beavers screened, and draw inferences on their adaptability to current living conditions.
- To determine beaver species, genetic diversity and relatedness of the beavers sampled.
- To apply permanently individually identifiable tags.



## Main findings

- Five free-living beavers live trapped on the River Otter, Devon were health screened and appropriate samples collected for genetic analysis.
- No evidence of non-native *Echinococcus multilocularis* or Tularaemia was determined.
- All screened beavers displayed good body condition, had no physical abnormalities, displayed haematological values within normal ranges and tested negative to all significant diseases screened.
- From a health and body condition perspective there is no evidence that beavers screened are failing to cope in an English environment or are suffering from compromised welfare.
- All beavers screened were physically and genetically confirmed as Eurasian beavers.
- Values of genetic diversity in the River Otter population were lower than for these possible source populations.
- Examination of genetic relatedness revealed that all beavers were closely related, consistent to belonging to a single family group. There is at least one sire absent from the family group that has not been sampled.
- All beavers were permanently identified with micro-chips. Ear tags were applied and individual tail scarring and notching patterns recorded.

# Health Screening

## MAIN FINDINGS

- Five free-living beavers live trapped on the River Otter, Devon were health screened and appropriate samples collected for genetic analysis.
- No evidence of non-native *Echinococcus multilocularis* or Tularaemia was determined.
- All screened beavers displayed good body condition, had no physical abnormalities, displayed haematological values within normal ranges and tested negative to all significant diseases screened.
- Bacteriology and parasitology presented no cause for concern.
- From a health and body condition perspective there is no evidence that beavers screened are failing to cope in an English environment or are suffering from compromised welfare.
- All beavers were permanently identified with micro-chips. Ear tags were applied and individual tail scarring and notching patterns recorded.

## METHODS

The disease screening protocol for live beavers was based on that already established for the official Scottish Beaver Trial<sup>1</sup>, and those undertaken for wild beavers living on the Tay and Earn catchments, of unknown origin, undertaken on behalf of Scottish Natural Heritage<sup>2</sup>.

All beavers were anaesthetised using 4% isoflurane in 100% oxygen via a face mask and maintained on 1.5-2% isoflurane in 100% oxygen.

A full physical examination was undertaken whilst anaesthetised, including:

- eyes – symmetry of head, eyes for ocular discharge
- ears – check for parasites
- nose – check for nasal discharge, abrasions
- teeth – check for malocclusion, signs of dental disease, abdominal wear, trap injuries
- integument (including tail and feet) – check for wounds, ectoparasites, dermatitis, condition of fur, covering of fat over the pelvic region, spine and tail was assessed
- tail – check for wounds, abrasions, thickness

Each beaver was assessed for scars or any signs of previous trauma, as well as for the presence of any external parasites. Palpation was performed of all the limb joints to ensure normal range of motion, along with an abdominal palpation for any organ enlargements or abnormal masses. Fur condition was assessed as lack of proper grooming may represent underlying health issues and poorer body condition.

Weight was measured and body score assessed according to the standard rodent body scoring system. Each beaver was scanned for the presence of an identity microchip, and if not present beavers were microchipped in the inter-scapular region to allow future identification.

Sex was initially established through the examination of the colour and viscosity of the anal gland secretions (AGS). The sex of each individual was further confirmed through laparoscopic examination. Blood was taken aseptically from the ventral tail vein for diagnostic testing and an additional sample taken for genetic screening. Haematology and serum biochemistry were performed as a general assessment of each beaver's general state of health (SAC Consulting Veterinary Services, Scotland's Rural College).

Further specific serological testing was performed as follows: European *Leptospira* serovars using the microscopic agglutination test (MAT) (Animal Health Veterinary Laboratory Agency, Weybridge); *Echinococcus multilocularis* by means of two different enzyme-linked immunosorbent assays (ELISAs) targeted against the EM 18 and EM 2 antigens, used for human *EM* diagnosis, as well as a recently developed immunoblot. A specific anti-beaver IgG conjugate was used for testing at University of Bern, Switzerland. Polymerase chain reaction (PCR) testing was also carried out for tularaemia on serum (National Veterinary Institute, Norway).

Faeces were taken directly from the beaver's rectum, and rectal microbiology swabs were taken. Faecal samples underwent flotation with saturated salt solution for nematodes and sedimentation for trematodes, as well as microscopy for coccidia, *Cryptosporidium* spp., and *Giardia* spp. Standard microbiological culture for bacterial enteric pathogens, including enriched media for *Salmonella* was performed (SAC Consulting Veterinary Services, Scotland's Rural College). In addition, a bronchoalveolar lavage was performed for testing for bovine tuberculosis (*Mycobacterium bovis*), although the disease has not been reported in beavers. Lavage fluid was submitted for standard mycobacterial culture and examined cytologically, including acid-fast staining for acid fast/mycobacterial organisms (Veterinary Pathology, RDSVS, University of Edinburgh).

An abdominal ultrasound examination was performed, with specific attention to the liver, to detect any abnormalities that could be indicators of *EM*. A 2-5MHz frequency convex abdominal ultrasound probe was used, and examinations recorded on a digital video recorder. Ultrasonography was performed by wetting the dense fur with 90% ethanol to allow adequate contact and good visualisation, in preference to clipping of fur, which it was considered may adversely affect the beavers waterproofing and thermal insulation when returned to the wild after testing.

A minimally invasive laparoscopic examination of the abdominal cavity was performed in the four adult animals to assess the liver and abdominal viscera for any signs of *EM* or other pathology not evident on ultrasonography, and physical examination. The fur and skin in the ventral midline region of the umbilicus was thoroughly cleaned and disinfected with a dilute chlorhexidine or dilute povidone/iodine based surgical scrub, followed by the application of surgical ethanol. A 6mm skin incision was made and the underlying ventral muscles bluntly dissected to allow open access placement of a blunt trocar and 5mm cannula. The abdomen was insufflated with 8-10mmHg pressure using medical grade carbon dioxide. A five millimetre, 30 degree, 30cm paediatric laparoscope was inserted and the abdomen fully examined, with specific attention to the liver. The animal was repositioned in left and right lateral recumbency to allow movement of the viscera, and visualisation of all organ surfaces. At the end of the minimally invasive laparoscopic

examination the abdomen was deflated and the cannula removed and the muscle and skin closed with absorbable monofilament Poliglecaprone suture material in two layers, the skin closure being performed with a buried absorbable intradermal suture placement. Tissue adhesive was applied to the small skin incision wound to aid in immediate post-operative waterproofing. The resultant wound was approximately twice the size of a microchipping wound.

## RESULTS

### PHYSICAL EXAMINATION

- Five individuals (two family units) were live trapped using Bavarian beaver traps by Animal and Plant Health Agency (APHA) staff, then housed for ~6 weeks maximum in captive facilities at Derek Gow Consultancy. On physical examination it was suspected that these individuals consisted of one adult female and male in one family unit, and one adult female and male, with a yearling individual in the another family unit.
- All animals were physically healthy and presented no obvious deformities, discharge or obvious signs of disease. Evidence of previously healed wounds on all adult tails, and the hind foot of one adult female were observed, indicating historic injuries, most likely as a result previous territorial disputes. This is common and to be expected for this species. The missing hind toe of the adult female could also be indicative of historic trapping and/or transportation injury.
- Sex confirmation, through examination of anal gland secretion and extended nipples, indicated that two adult females and two males were present, with the yearling sexed as female.
- Estimation of age class was made according to time of year, weight and body dimensions. The male 'adults' were considered to be smaller than the adult females, though of body dimensions that could classify then as adults or mature sub-adults as a minimum. Weights and body dimensions are given in Table 1.
- All beavers were deemed in good to very good body condition, with scores of 3 to 4, and defined as normal to good given time of year and age class. This was determined through examination of fat coverage of vertebrae and dorsal pelvis, and tail condition (thickness and lack of prominent tail arches).
- All beaver were tagged with passive transponder tags for individual identification (see appendix 1.).

### HAEMATOLOGY AND SERUM BIOCHEMISTRY

- These parameters were judged against previously established normal values for the Eurasian beaver<sup>3</sup>. All were largely unremarkable.
- No haemoparasites were recorded.

## ECHINOCOCCUS MULTILOCULARIS SCREENING

- The four adult individuals were screened for *EM* as described above and all were found negative on serology, ultrasound and laparoscopic examination

## ADDITIONAL DISEASE SCREENING

- All beavers were negative for Tularemia, as determined through PCR of serum samples.
- One beaver tested positive for Leptospirosis (*L. javanica*), the remaining four tested negative.
- On analysis of lung washes on the four adult beavers, all beavers were negative for acid fast/mycobacterial organisms, there was no evidence of any inflammatory lung response.
- Bacteriology screening for Salmonella and Johnes were negative.
- Parasitology was unremarkable with no evidence of cryptosporidium, Giardia or lungworm. Nematode and Coccidial oocyst counts were <50 and therefore below the detectable threshold. Fluke eggs were only detected in D1 in which atypical eggs were seen, which are most likely to be *Strichorchis subtriquetrus* (beaver intestinal fluke), with no fluke eggs detected in any of the remaining individuals.

## BODY CONDITION

### PHYSICAL EXAMINATION

BEAVER ID	SEX	AGE CLASS	WEIGHT (KG)	TAIL LENGTH (CM)	TAIL WIDTH	BODY LENGTH	TTA* (TAIL THICKNESS AT PT A)	TTB	TTC	TTD
<b>D1</b>	F	Adult	17.3**	33.5	16	87	4.6	2.2	1.3	0.8
<b>D2</b>	M	Adult /sub-adult	18.0	31	15	84	3.4	3.3	1.5	0.9
<b>D3</b>	F	Adult	23.5	30	14	86	4.1	3.3	1.5	0.8
<b>D4</b>	M	Adult /sub-adult	19.0	31	15	80	3.5	2.2	1.2	0.6
<b>D5</b>	F	Kit	~	~	~	~	~	~	~	~

\*point of each tail thickness measurements (A-D) displayed in Appendix 1.

\*\*likely underestimate due to animal movement during measurement.



**TABLE 1: BODY METAMORPHICS OF BEAVERS EXAMINED**

BEAVER ID	SEX	REPRODUCTIVE STATUS	BODY CONDITION	TAIL FEATURES	ADDITIONAL COMMENTS
<b>D1</b>	F	Pregnant	Good	Small nip lower right	Upper incisor recently chipped, potential trap injury. Back left webbing in foot split but healed
<b>D2</b>	M	N/A	Good	Split lower right, scar mid right	
<b>D3</b>	F	Pregnant	Very good	Split lower left, nip upper left, tip and mid right, scar mid left	Second upper toe on left hind foot missing, fresh multiple scales missing from underside of tail this is not a trapping injury, most likely caused by rough surfaces in captive environment
<b>D4</b>	M	N/A	Good	Split lower left	Split right ear, healed
<b>D5</b>	F	N/A	Good	Complete	Very reactive to handling, not anaesthetized so handling time minimised

**TABLE 2: REPRODUCTIVE STATUS AND BODY CONDITION.**

### INDIVIDUAL IDENTIFICATION

Individual passive identification tags were inserted subcutaneously in the dorsal neck region of each beaver to enable individual identification over the long-term.

Unique tail scarring and notching, indicative of previous territorial disputes and/or old injuries were recorded on all of the beavers, except the kit (tail completely intact). These marking were recorded (Appendix 1), and could be used for individual identification.

<sup>1</sup> Goodman G, Girling S, Pizzi R, Rosell F & Campbell-Palmer R. 2012. Establishment of a health surveillance program for the reintroduction of the Eurasian beaver (*Castor fiber*) into Scotland. *Journal of Wildlife Disease* 48: 971-978.



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<sup>2</sup>Campbell-Palmer R, Pizzi R, Dickinson H & Girling S. 2015. Trapping and health screening of free-living beavers within the Tayside catchment, east Scotland. Scottish Natural Heritage Commissioned Report 681.

<sup>3</sup>Girling S, Campbell-Palmer R, Pizzi R, Fraser MA, Cracknell J, Arnemo J, & Rosell F. Haematology and serum biochemistry parameters and variations in the Eurasian beaver (*Castor fiber*). PLoS ONE (in press).

# Genetic Screening

## MAIN FINDING

- All five beavers screened were genetically determined as being Eurasian beaver (*Castor fiber*).
- All five samples were most likely to be of German reintroduced population origin and had were highly genetically similar to reference samples held for Bavaria and Baden-Württemberg populations. Values of genetic diversity in the River Otter population were lower than for these possible source populations.
- Examination of genetic relatedness revealed that all beavers were closely related, consistent to belonging to a single family group. It was not possible to be certain of the exact pattern of relatedness between the animals because they were all so closely related, but it was possible to make a number of statements excluding certain relationships and suggest a most likely arrangement of relatedness. There is at least one sire absent from the family group that has not been sampled.
- Samples were stored for any further DNA analysis.

## METHODS

- DNA was extracted using a standard QIAGEN kit and normalised to 10ng/μl. Samples were run with a test developed at the WildGenes Laboratory of the Royal Zoological Society of Scotland that consists of two mitochondrial Single Nucleotide Polymorphism markers (SNPs) that discriminate between *C. fiber* and *C. canadensis*. SNP analysis was conducted using an Applied Biosystems StepOne real-time thermal cycler and followed the standard amplification conditions for KASPar SNP probes as recommended by the manufacturer and as previously detailed<sup>4</sup>.
- The samples were run alongside two negative controls and positive controls for the two species (*C. fiber*, *C. canadensis*) in the following PCR plate set up (Table 3).

## SPECIES TESTING

- The beavers all clearly clustered genetically with *C. fiber* (Table 3). One sample (BEV727) failed at one of the markers, however it clustered clearly with *C. fiber* at the second marker. Low DNA concentration is this sample is the likely cause of failure for this first SNP.
- Details of the genetic clustering plots can be found in Appendix 2.

TABLE 3: PLATE LAYOUT FOR SAMPLES TESTED INCLUDING THE NEGATIVE AND POSITIVE CONTROLS. LAYOUT CAN BE COMPARED TO THE RESULTS IN APPENDIX 1.

	1	2	3	4	5	6	7	8
<b>A</b>	Negative control	Negative control	BEV727 River Otter sample 1	BEV728 River Otter sample 2	BEV729 River Otter sample 3	BEV730 River Otter sample 4	BEV731 River Otter sample 5	BEV018 Eurasian Control Norway
<b>B</b>	BEV019 Eurasian Control Norway	BEV094 Canadian control	BEV095 Canadian control	BEV096 Canadian control	BEV097 Canadian control	BEV039 Eurasian control Bavaria	BEV250 Eurasian control Belarus	BEV373 Eurasian Control Norway

#### POPULATION ORIGIN AND GENETIC DIVERSITY

- Samples were run with a test developed at the WildGenes Laboratory of the Royal Zoological Society of Scotland that consists of 29 nuclear Single Nucleotide Polymorphism markers (SNPs) that discriminate between different populations of beaver<sup>2</sup>. SNP analysis was conducted using an Applied Biosystems StepOne real-time thermal cyclers and followed the standard amplification conditions for TaqMan SNP probes as recommended by the manufacturer. Samples were run with 2 negative and 7 positive controls.
- Samples were compared to a reference dataset of 307 beavers of known population origin<sup>3</sup> using the population assignment program GenClass2.
- All animals assigned with high probability to either Bavarian or Baden-Württemberg populations (Figure 1). These are German populations of mixed reintroduced origin- for further details see<sup>3</sup>. This strongly suggest that the beavers came from German mixed reintroduced stock. The animals assign with very low probability to France, Norway and a number of Eastern European/ Eurasian populations.
- The animals had a lower level of heterozygosity (He) than the reference source populations that they matched to. The value of He for Devon was 0.339 (with a standard error of 0.03) compared to values of 0.453 (s.e. 0.016) and 0.478 (s.e. 0.005) for Baden-Württemberg and Bavaria respectively (values calculated using comparative loci).

<sup>2</sup> Senn H, Ogden R, Frosch C, Syrůčková A, Campbell-Palmer R, Munclinger P, Durka W, Kraus R H S, Savejev A P, Nowak C, Stubbe A, Stubbe M, Michaux J, Lavrov V, Samiya R, Ulevicius A, & Rosell F, 2014, Nuclear and mitochondrial genetic structure in the Eurasian beaver (*Castor fiber*) – implications for future reintroductions. *Evolutionary Applications* 7 645-662, *Evolutionary Applications* 7 645-662

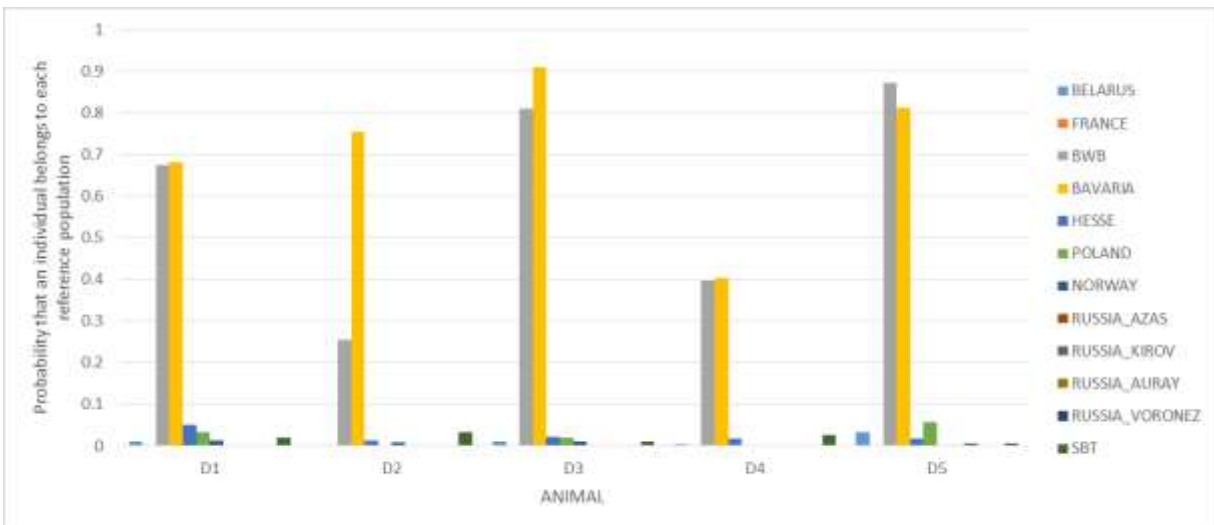


FIGURE 1: THE PROBABILITY THAT EACH INDIVIDUAL BELONGS TO A NUMBER OF REFERENCE POPULATIONS (FOR DETAILS OF THE POPULATIONS SEE SENN ET AL. 2014), BWB IS BADEN-WÜRTTEMBERG.

### FAMILY RELATIONSHIPS

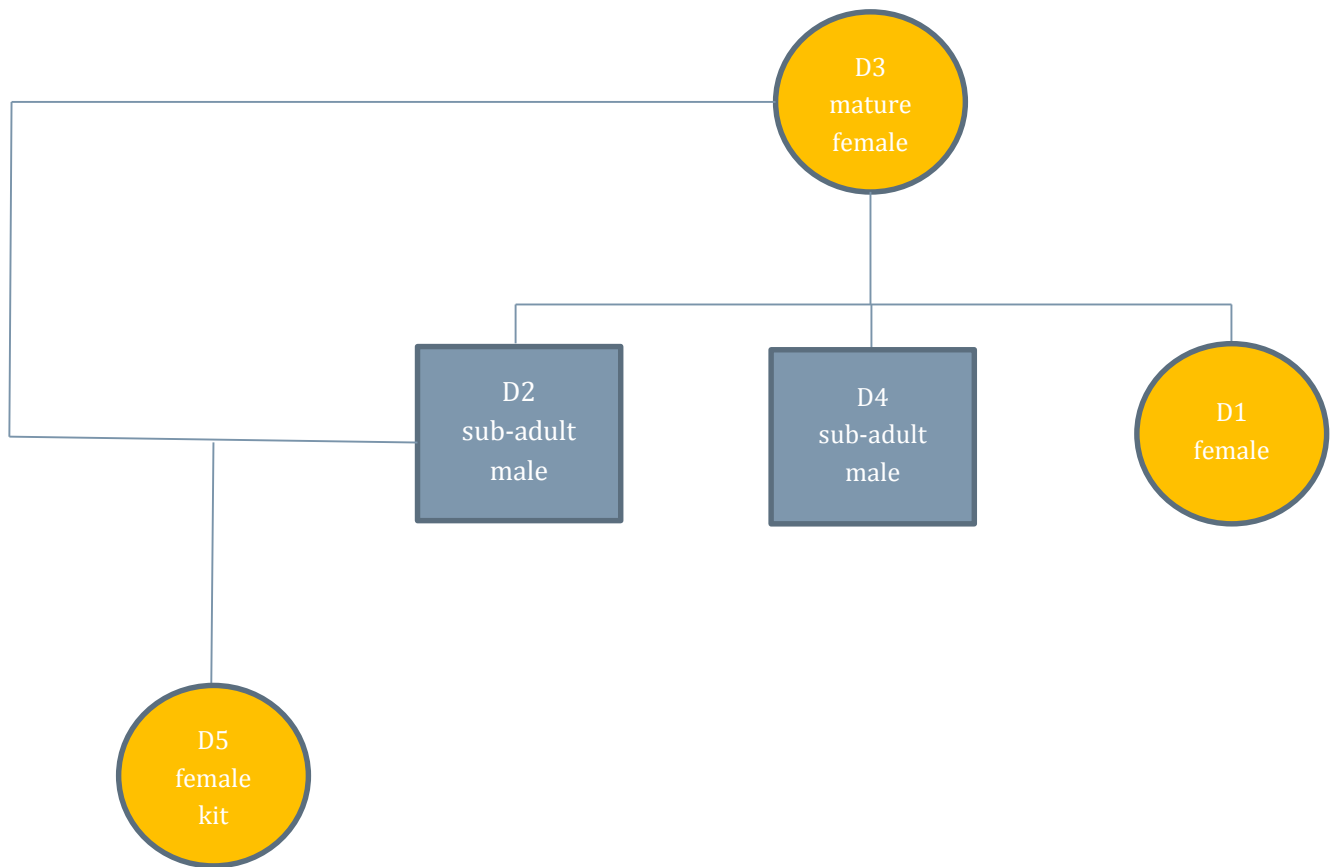
- Using the same 29 nuclear Single Nucleotide Polymorphism markers (SNPs) as used for population assignment, potential familial relationships were examined by calculating estimates of pairwise molecular relatedness (in the software Genalex). Likely family combinations were also examined by eye to exclude possible combinations and their statistical likelihood examined in the software Colony. These markers have been shown to have sufficient power to examine issues of relatedness in the target populations<sup>3</sup>.
- Pair-wise relatedness between all individuals was high (Table 4) and was approximately equivalent to being between the first order (e.g. parent-offspring/full sib) and the second order (e.g. half-sib) relationship level. There is a lot of statistical noise around these estimates so it is not possible to use these estimates to be sure of the exact degree of relatedness (other than that is very close).
- Examination of the genotypes involved in the different possible parent-offspring relationships (that were likely between the beavers based on their age and sex) was able to rule out or suggest a number of potential parent-offspring combinations (Table 5). None of the potential relationships could be confirmed with a high degree of statistical certainty due to close degree of relatedness of the individuals (i.e. another form of close relationship is also theoretically possible). The most likely arrangement is given in Figure 2.

TABLE 4 & 5: LYNCH & RITLAND PAIRWISE RELATEDNESS ESTIMATES FOR ALL POSSIBLE PAIRWISE RELATIONSHIPS, ARE EQUIVALENT TO BEING BETWEEN THE FIRST ORDER (E.G. PARENT-OFFSPRING/FULL SIBLINGS 0.5) AND THE SECOND ORDER (HALF-SIB 0.25) RELATIONSHIP LEVEL FOR ALL INDIVIDUALS.

COMBINATION		LYNCH & RITLAND 1999 PAIRWISE RELATEDNESS
<b>D1</b>	<b>D2</b>	0.278
<b>D1</b>	<b>D3</b>	0.213
<b>D1</b>	<b>D4</b>	0.315
<b>D1</b>	<b>D5</b>	0.279
<b>D2</b>	<b>D3</b>	0.271
<b>D2</b>	<b>D4</b>	0.306
<b>D2</b>	<b>D5</b>	0.296
<b>D3</b>	<b>D4</b>	0.286
<b>D3</b>	<b>D5</b>	0.386
<b>D4</b>	<b>D5</b>	0.343

COMBINATION	SEX/AGE	RELATIONSHIP
<b>D3 &amp; D5</b>	<b>Adult F &amp; Yearling</b>	D3 potential mother of D5
<b>D1 &amp; D5</b>	<b>Adult F &amp; Yearling</b>	D1 not mother of D5
<b>D2 &amp; D5</b>	<b>Male &amp; Yearling</b>	D2 potential sire of D5
<b>D4 &amp; D5</b>	<b>Male &amp; Yearling</b>	D4 not sire of D5
<b>D1 &amp; D2</b>	<b>Adult F &amp; Male</b>	D1 not the mother of D2
<b>D1 &amp; D4</b>	<b>Adult F &amp; Male</b>	D1 not the mother of D4
<b>D3 &amp; D2</b>	<b>Adult F &amp; Male</b>	D3 potential mother of D2
<b>D3 &amp; D4</b>	<b>D4</b>	D3 potential mother of D4
<b>D3 &amp; D1</b>	<b>Adult F &amp; Adult F</b>	D3 potential mother of D1

FIGURE 2: POTENTIAL PEDIGREE RELATIONSHIP OF SAMPLED ANIMALS. MOST LIKELY CONFIGURATION BASED ON AGE AND GENOTYPE OF BEAVERS. NOTE THAT THE POTENTIAL SIRES OF D2, D4 AND D1 IS ABSENT.



# Conclusions

## HEALTH SCREENING

All individuals were passed fit for re-release, presenting no health concern to humans, livestock or other wildlife.

All individuals were free of *Echinococcus multilocularis*, Tularemia, Johnes disease and bovine TB.

All beavers were deemed free from of the ectoparasite, beaver beetle. Eggs of the intestinal beaver fluke was most likely detected in one adult female. This is a host specific parasite, with an intermediate stage in aquatic snail species, present in other free-living beavers in Britain and commonly recorded in all beaver populations and reintroductions across Europe.

All individuals were in good to very good body condition given age class and time of year, and so appear to be adapting well to English landscapes and present no welfare cause for concern. Evidence of previous injuries, most likely normal territorial disputes, are present but these are healed and of no cause of concern.

The two adult females were determined to be pregnant.

The two males were smaller than the adult females, though both sexually mature and theoretically capable of reproduction. However, the second male examined was radiographed, and determined to be skeletally immature as growth plates of long bones, although almost closed, were still open. This leaves the possibility of this individually being an older sub-adult and not conclusively being the dominant and hence the reproducing male in this family unit.

## GENETIC SCREENING

All individuals were genetically determined as Eurasian beavers. All five samples were most likely to be of German reintroduced population origin and had were highly genetically similar to reference samples held for Bavaria and Baden-Württemberg populations. Values of genetic diversity in the River Otter population were lower than for these possible source populations.

Examination of genetic relatedness revealed that all beavers were closely related, consistent to belonging to a single family group. It was not possible to be certain of the exact pattern of relatedness between the animals because they were all so closely related, but it was possible to make a number of statements excluding certain relationships and suggest a most likely arrangement of relatedness. There is at least one sire absent from the family group that has not been sampled.

## WELFARE STATUS

Although there was evidence of previous injuries, there were no welfare causes for concern. Body condition, reproductive status and evidence of successful reproduction with offspring in good body and health condition, means there are no obvious issues with the beavers' ability to survive and successfully adapt in their current living conditions.





## INDIVIDUAL IDENTIFICATION

Individual passive identification tags were applied to each beaver, so that each could be permanently identified with a micro-chip reader.

Previous scarring and injury patterns were recorded that could enable individual identification, particularly for future camera trapping work for example.



## Acknowledgements

Screening work for this project would not have been possible without the trapping assistance from APHA, specifically Julia Coats and Dave Parrot. Thanks to Derek Gow Consultancy for captive care of these animals, particularly Becky for all her hard work and organisation. Many thanks to Donna Brown RVN CertVNES for her vital assistance in the health screening process, in particular anaesthetic care of all the beavers. Genetic analysis was undertaken by Royal Zoological Society of Scotland WildGenes, with laboratory work undertaken by Jennifer Kaden. Devon Wildlife Trust staff, in particular Mark Elliott and Pete Burgess, for all their assistance in their facilitation of this work. Funding for this work was provided by DEFRA, Devon Wildlife Trust and RZSS.

# Appendix


Appendix 1. Copy of individual trapping and handling sheets for each of the five individuals screened.

**LIVE-TRAPPING OF BEAVERS – FIELD SHEET**

Date of capture: 8/3/15 Trap: PEN 1 Transported: N/A

ID of trapped animal (ANZIS ID/trapping code/track): E2322 E2323

Locality: DEWON ONG

Barcode: 

Time removal from trap:  Time at start of handling: 10:57 <sup>Start 11:00</sup> Time anaesthetic: 11:03

Body length – nose to base of tail (following spine): 87 cm

Tail length: 33.5 & width 16 cm. Tail thickness at a 4.66 b 2.17 c 1.25 d 0.80 cm  
(see diagram of tail for location). Tail thickness taken on left  right  (cross in correct box)

Tail scars and notches on the sides R. & on top Yes (sketch scars on diagram below)

Samples taken  <sup>Subs.</sup>

Hair  faeces x2  castoreum  AGS (anal gland secretion)  tissue  Blood

EM screening:  Genetic sample:  Sex: ♀

Eartag in left ear (type, colour + number):

Eartag in right ear (type, colour + number):

PIT code: No PIT TAG

Size of nipples: 0.5 cm

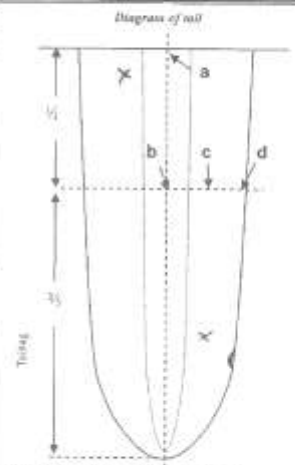
Weight of beaver: 17.3 kg

Time of crating: 12:28 Recovery:

Time of release:

General comments  
WPSR 10022R C14, P050  
BACH LEFT WEBBIAN SPHIT  
pregnant

Diagram of tail



Note location of tail-tag - show tail scars

LIVE-TRAPPING OF BEAVERS – FIELD SHEET

Date of capture  Trap  Transported   
 ID of trapped animal (Animal ID/trapping code/name)   
 Locality   
 Time removal from trap  Time at start of banding  <sup>Start 12:45</sup> Time anaesthetic

Body length – nose to base of tail (following spine)  cm  
 Tail length  & width  cm. Tail thickness at a  b  c  d  cm  
 (see diagram of tail for location). Tail thickness taken on left  right  (cross in correct box)  
 Tail scars and notches on the sides  & on top  (sketch scars on diagram below)

Samples taken  X  
 Hair  faeces x2  castoreum  AGS (anal gland secretion)  tissue  Blood   
 EM screening  Genetic sample  Sex  MALE

Eartag in left ear (type, colour + number)   
 Eartag in right ear (type, colour + number)

PIT code    
 941000017319848

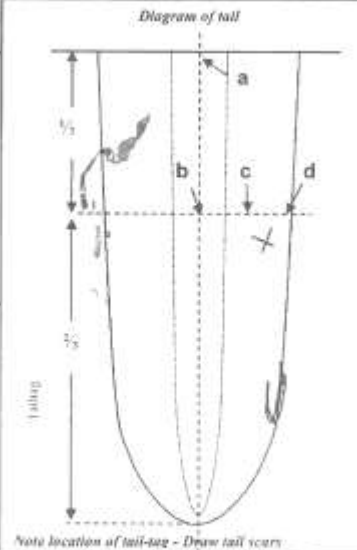
Size of nipples  cm

Weight of beaver  kg

Time of crating  *Recover*

Time of release

General comments



LIVE-TRAPPING OF BEAVERS – FIELD SHEET

Date of capture 8/3/15 Trap PEN2 Transported N/A

ID of trapped animal (Animal ID/trapping order name) FE2309

Locality DEVON 3  
RIGHT YELLOW

Time removal from trap  Time at start of handling 14:25 Time anaesthetic 14:28

Body length – nose to base of tail (following spine) 86 cm

Tail length 30 & width 14 cm. Tail thickness at a 4.08 b 3.32 c 1.5 d 0.8 cm

(see diagram of tail for location). Tail thickness taken on left  right  (cross in correct box)

Tail scars and notches on the sides  & on top  (sketch scars on diagram below)

Samples taken

Hair  faeces x2  castoreum  AGS (anal gland secretion)  tissue  Blood

EM screening  Genetic sample  Sex ♀

Eartag in left ear (type, colour + number)

Eartag in right ear (type, colour + number)

PIT code 98100000390815

Size of nipples 1cm. cm

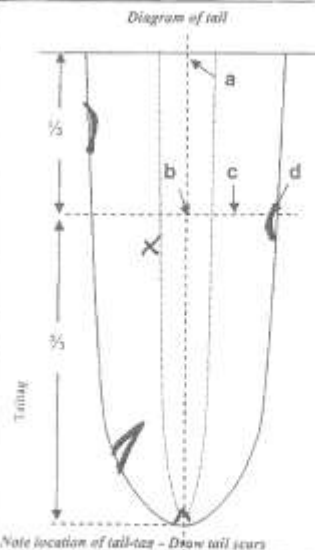
Weight of beaver 23.5 kg

Time of crating 15:58 Recovery

Time of release

General comments

Left toe missing. (second toe)  
PIT TAG FOUND  
multiple scales missing - froth  
underside - not trap injury  
Stiles were on lower wires.



LIVE-TRAPPING OF BEAVERS – FIELD SHEET

Date of capture 2/3/15 Trap Pen 2 Transported

ID of trapped animal (Animal ID+trapping code+name): OSLON 4  84100017319847

Locality REZ LPI  
Left

Time removal from trap  Time at start of handling 16:08 Time anaesthetic 16:11

Body length – nose to base of tail (following spine) 80 cm

Tail length 31 & width 15 cm. Tail thickness at a 3.5 b 2.21 c 1.21 d 0.6 cm  
(see diagram of tail for location). Tail thickness taken on left  right  (cross in correct box)

Tail scars and notches on the sides  & on top  (sketch scars on diagram below)

Samples taken   
Hair  faeces x2  castoreum  AGS (anal gland secretion)  tissue  Blood   
EM screening  Genetic sample  Sex ♂

Eartag in left ear (type, colour + number)

Eartag in right ear (type, colour + number)

PIT code

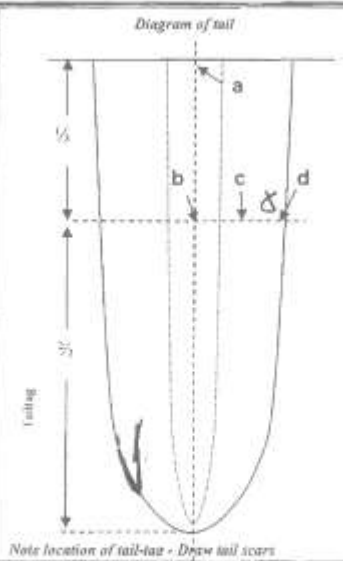
Size of nipples N/A cm

Weight of beaver 19 kg

Time of crating 17:32 Recovery

Time of release

General comments  
Spit ear on right-headed



### LIVE-TRAPPING OF BEAVERS – FIELD SHEET

Date of capture 8/3/15 Trap Par 2 Transported   
 ID of trapped animal (Animal ID + trapping code + name) Small blue Cg  
 Locality Yearling E210 Devon 5

Time removal from trap  Time at start of handling  Time anaesthetic

Body length – nose to base of tail (following spine)  cm  
 Tail length  & width  cm. Tail thickness at a  b  c  d  cm  
 (see diagram of tail for location). Tail thickness taken on left  right  (cross in correct box)  
 Tail scars and notches on the sides  & on top  (sketch scars on diagram below)

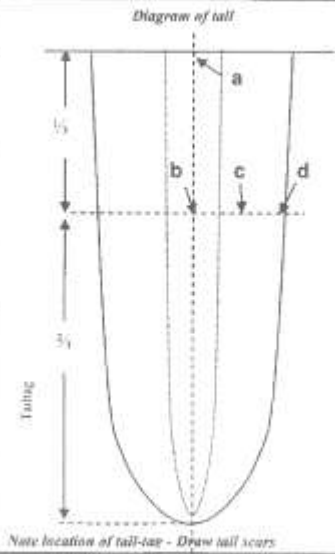
Samples taken   
 Hair  faeces x2  castoreum  AGS (anal gland secretion)  tissue  Blood   
 EM screening N/A Genetic sample  Sex ♀

Eartag in left ear (type, colour + number)   
 Eartag in right ear (type, colour + number)

PIT code    
 941000017319887

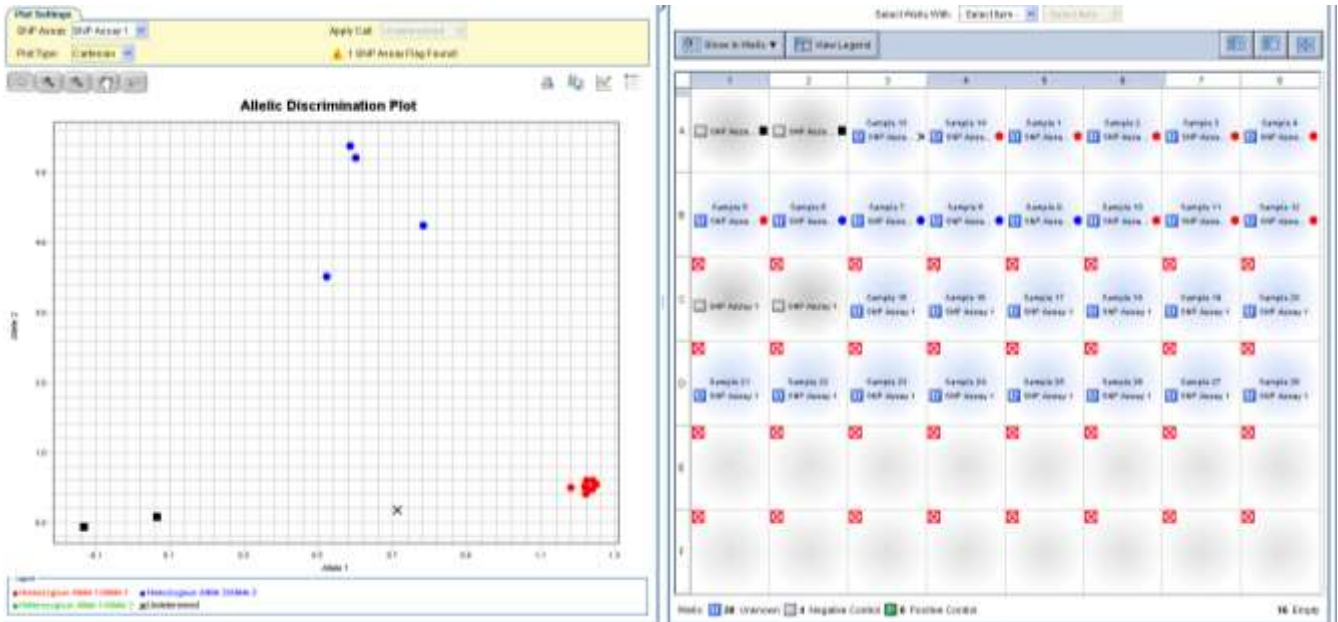
Size of nipples  cm  
 Weight of beaver  kg  
 Time of crating   
 Time of release

General comments



Appendix 2. Screenshots of the genetic clustering results for each of the two SNPs tested. The results for each sample is given on the right hand side and can be compared to the plate set-up in Table 3. The colouration of each point represents its genetic origin : Blue = *C. canadensis*, Red = *C. fiber*. Black=negative control, "x"= sample failed.

SNP1



SNP2



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