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► **To cite this version:**

P Chevret, Lionel Hautier, Guila Ganem, Jeremy Herman, Sylvie Agret, et al.. Genetic structure in Orkney island mice: isolation promotes morphological diversification. *Heredity*, 2021, 126 (2), pp.266-278. 10.1038/s41437-020-00368-8 . hal-02950610

**HAL Id: hal-02950610**

**<https://cnrs.hal.science/hal-02950610>**

Submitted on 23 Nov 2020

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1 **Genetic structure in Orkney island mice: isolation promotes morphological diversification**

2

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20 **Running title:** Evolution of Orkney mice

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22 **Word count:** 5605

23

24 **Abstract**

25

26 Following human occupation, the house mouse has colonized numerous islands, exposing the species  
27 to a wide variety of environments. Such a colonization process, involving successive founder events  
28 and bottlenecks, may either promote random evolution or facilitate adaptation, making the relative  
29 importance of adaptive and stochastic processes in insular evolution difficult to assess.

30 Here, we jointly analyse genetic and morphometric variation in the house mice (*Mus musculus*  
31 *domesticus*) from the Orkney archipelago. Genetic analyses, based on mitochondrial DNA and  
32 microsatellites, revealed considerable genetic structure within the archipelago, suggestive of a high  
33 degree of isolation and long-lasting stability of the insular populations. Morphometric analyses,  
34 based on a quantification of the shape of the first upper molar, revealed considerable differentiation  
35 compared to Western European populations, and significant geographic structure in Orkney, largely  
36 congruent with the pattern of genetic divergence. Morphological diversification in Orkney followed a  
37 Brownian motion model of evolution, suggesting a primary role for random drift over adaptation to  
38 local environments. Substantial structuring of human populations in Orkney has recently been  
39 demonstrated, mirroring the situation found here in house mice. This synanthropic species may thus  
40 constitute a bio-proxy of human structure and practices even at a very local scale.

41

42

## Evolution of Orkney mice

43

44 **Introduction**

45

46 Islands are well-known “laboratories of evolution” suited to investigation of processes of divergence  
47 (Berry, 1996). The paradigm examples of insular evolution involve dramatic changes in body size,  
48 with dwarfism of large species and gigantism of small species (Lomolino, 1985), but morphological  
49 differentiation and radiation are also frequent [e.g. (Losos and Ricklefs, 2009)]. Such cases of extreme  
50 evolution are mostly ascribed to adaptation to local ecological conditions, in particular the release  
51 from predation and interspecific competition (Lomolino *et al*, 2012). Random evolution is also an  
52 important driver of morphological differentiation on islands, because of the large effect of genetic  
53 drift in the small founding propagules and in the subsequent isolated populations (Sendell-Price *et al*,  
54 2020). A way to disentangle adaptive and random factors, and hence to better assess the contextual  
55 driving forces of insular evolution, may be to consider the relationship between genetic and  
56 morphological markers: random morphological evolution should be coupled to, and adaptive  
57 evolution uncoupled from, neutral genetic divergence (Polly, 2004; Renaud *et al*, 2007).

58 The Orkney archipelago, lying close to the northern coast of mainland Scotland, is well known for its  
59 wealth of Neolithic sites. By this period, ca. 5,000 years BP, the long-tailed field mouse (*Apodemus*  
60 *sylvaticus*) and the common vole (*Microtus arvalis*) had been introduced to the archipelago  
61 (Romaniuk *et al*, 2016). The Orkney vole is recognisable by its large size and characteristic tooth  
62 shape (Cucchi *et al.*, 2014). Localised morphological diversification, echoed in the genetic structure,  
63 also occurred between islands of the archipelago (Martínková *et al*, 2013). The congruent patterns of  
64 divergence in molar shape and neutral molecular markers suggest a primary role for drift in the  
65 process of Orkney vole morphological evolution (Cucchi *et al.*, 2014).

66 The Western European house mouse (*Mus musculus domesticus*) was first introduced to Orkney  
67 about 4,000 years later. The Orkney islands had a central position within the Norwegian Viking

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68 kingdom, which was active from the late 8<sup>th</sup> to the 11<sup>th</sup> centuries AD. The house mouse was  
69 introduced as an unintentional stowaway during this period of intense maritime traffic (Searle et al.,  
70 2009). Despite the relatively short period of time since their introduction to Orkney, house mice  
71 display considerable diversity in tooth shape on the archipelago (Ledevin *et al*, 2016). The house  
72 mouse arrived on Orkney around the same time as potential predators such as the domestic cat, the  
73 red fox and the black rat (Cucchi *et al*, 2014). It was therefore confronted by ecological conditions  
74 similar to those encountered on the continent, a situation which is less prone to drive accelerated  
75 morphological divergence than would be the case when arriving in an insular location with a  
76 depauperate fauna (van der Geer *et al*, 2013). This should have mitigated the role of adaptation in  
77 the evolution of Orkney mice compared with continental relatives. As a synanthropic species, house  
78 mice are frequently translocated by humans (Cucchi, 2008; García-Rodríguez *et al*, 2018), possibly  
79 limiting the impact of founder effect and subsequent isolation.

80 Samples from house mouse populations on different Orkney islands were investigated for variation  
81 in mitochondrial DNA and at microsatellite loci, together with a geometric morphometric analysis of  
82 their molar shape. The genetic and morphological diversity of Orkney mice, and its relationship with  
83 Western Europe, were assessed, with particular emphasis on the comparison between morphological  
84 and molecular evolution. The main aims were firstly to identify the degree of differentiation between  
85 mice from Orkney and the continent, and between mice from within the Orkney archipelago; and  
86 secondly to determine the relative role of adaptation and drift in this differentiation. An accelerated  
87 morphological evolution on Orkney, compared to the genetic divergence, would point to a prime role  
88 of adaptation (Renaud *et al*, 2017), likely to occur in the peculiar insular conditions (Millien, 2006). In  
89 contrast, a primary role for drift would be indicated by morphological evolution paralleling genetic  
90 divergence, according to a Brownian model of evolution.

91

92

## 93 **Material and Methods**

### 94 *Sampling*

95 Mice were trapped during two field trips to the Orkney archipelago (Fig. S1). The islands of Eday,  
96 Faray, Papa Westray, Sanday and Westray were sampled in 1992 and Papa Westray, Burray, South  
97 Ronaldsay and Mainland were sampled in 2012 (Fig. S1B; Table S1). The sole mouse from Burray was  
98 grouped with mice from South Ronaldsay because the two islands are physically connected by a  
99 narrow isthmus.

100 Most mice were kept for several months in captivity before sacrifice (Ganem, 1998; Souquet *et al*,  
101 2019). All mice were sacrificed according to the directive 2010/63/UE of the European Parliament on  
102 the protection of animals used for scientific purposes. Skulls were manually prepared and stored at  
103 the Institut des Sciences de l'Evolution (Montpellier, France).

104 303 mice from Orkney were included in the morphometric analyses. 279 mice were genotyped at 19  
105 microsatellite loci and 79 mice were sequenced for the mitochondrial D-loop.

106

107 *Comparison between Orkney and continental populations.* – For the comparison of Orkney mice with  
108 continental populations from Western Europe, our 79 D-loop sequences were combined with  
109 sequences retrieved from GenBank. A first dataset included D-loop sequences from various origins in  
110 order to insert Orkney mice into a large phylogeographic context. A second dataset was compiled  
111 with sequences from the same localities as the morphometric sampling (Fig. S1A, Table S2).

112 The corresponding morphometric analysis included 593 mice from Orkney, the adjacent Scottish  
113 mainland and various continental populations (Fig. S1A; Table S2). Since body weight data were only  
114 available for a few continental populations, and given that most of the Orkney mice aged in  
115 laboratory conditions, a comparison of body size between continental and Orkney mice was not  
116 performed.

117

## Evolution of Orkney mice

118 Genetic and morphometric variation in Orkney. – A second set of analyses were devoted to the  
119 geographic structure within the Orkney archipelago; three markers were considered. (1) D-loop  
120 sequences. (2) Microsatellite data. (3) A morphometric analysis of molar shape, focused on mice that  
121 were also genotyped for microsatellites. This dataset included 268 mice (Table 1, Table S1). Given its  
122 extent, the island of Mainland was divided into discrete areas: North West (NW), North Central (NC),  
123 North East (NE), Central A5 and A7 localities (C57), Kirkwall (KIRK), South East (SE) and Deerness (DE).

124

125 *Molecular analyses*

126 Data acquisition. – DNA was extracted, amplified and aligned using standard protocols  
127 (Supplementary Text). The 79 new D-loop sequences were submitted to EMBL: accession numbers  
128 LR862585 to LR862663.

129 Nineteen microsatellite loci were selected based on previous studies (Britton-Davidian *et al*, 2017;  
130 Hardouin *et al*, 2010); genotyping and scoring were done according to standard procedures  
131 (Supplementary Text). 279 mice from the Orkney archipelago were successfully genotyped.

132 Phylogenetic analyses of D-loop sequences (for details, see Supplementary Text). – Three different  
133 datasets were analysed.

134 (1) *Inserting Orkney mice within the phylogeny of the house mouse.* The corresponding dataset  
135 included 1812 D-loop sequences of *Mus musculus domesticus* and two sequences each of *M.*  
136 *musculus castaneus* and *M. musculus musculus* used as outgroups. The final alignment  
137 comprised 728 haplotypes and 898 sites. The phylogenetic tree was reconstructed using  
138 MrBayes (Ronquist *et al*, 2012); robustness of the nodes was estimated with Posterior  
139 Probabilities (PP).

140 (2) *A D-loop dataset for comparison with the morphometric analysis at the European scale.* This  
141 dataset was designed to include only sequences matching the morphometric sampling (Table  
142 S2). It comprised 414 D-loop sequences and 879 positions. A neighbour-joining phylogeny

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143 was reconstructed based on p-distance estimated with MEGA 7 (Kumar *et al*, 2016).  
144 (3) *D-loop based genetic structure on the Orkney archipelago*. The 79 new D-loop sequences  
145 were combined with 67 sequences retrieved from previous studies. The final alignment  
146 comprised 146 sequences and 879 nucleotides. Haplotypes and nucleotide diversity indices  
147 were determined for each island with DNAsp v6 (Rozas *et al*, 2017) and Arlequin 3.5  
148 (Excoffier and Lischer, 2010). A haplotype network was inferred using the median-joining  
149 algorithm as implemented in POPART (Leigh and Bryant, 2015). As three of the 17 haplotypes  
150 were characterized by indels that are not taken into account by PopART, we coded these  
151 indels as new sites with A(deletion)/T(insertion) at the end of the alignment.

152

153 *Population genetic analyses based on microsatellite data*

154 Genetic diversity. – The genetic diversity within each island of the Orkney archipelago was  
155 characterised , using Arlequin 3.5 (Excoffier and Lischer, 2010), by the mean number of alleles per  
156 locus (A), expected (He) and observed (Ho) heterozygosities. The differentiation between Orkney  
157 islands and populations of Mainland was estimated using pairwise Fst. Significance was tested using  
158 1000 permutations.

159 Population structure. – The microsatellite data were first analysed using STRUCTURE 2.3.4 (Pritchard  
160 *et al*, 2000). This method failed to provide consistent results, providing K estimates that greatly  
161 varied between each run. Therefore, we used a Discriminant Analysis of Principal Components  
162 (DAPC) (Jombart *et al*, 2010). Isolation by distance (IBD) on molecular distances was tested at the  
163 scale of the whole archipelago, and on Mainland only. The distance between locations “as the crow  
164 flies” (i.e. in straight line) was used in both cases, because mice may have benefitted from human-  
165 mediated transport on both land and sea.

166

167 *Data acquisition for morphometrics*

## Evolution of Orkney mice

168 The first upper molar (UM1) is known to be highly evolvable in the house mouse, especially on  
169 islands (Renaud *et al*, 2011) and was therefore chosen as the character of interest. Three-  
170 dimensional methods have been developed to characterize its occlusal geometry (Ledevin *et al*,  
171 2016). However, an outline-based 2D approach has been chosen here, for two reasons. Firstly, the  
172 2D approach provides a good approximation of the 3D signal (Ledevin *et al*, 2016) and, because of its  
173 reduced cost compared to 3D analyses based on  $\mu$ CT-scans, it allowed us to include all the available  
174 specimens in the morphometric study all. In addition, the 3D geometry of the molar is strongly  
175 impacted by wear. Most Orkney mice were kept in the laboratory for some time, allowing them to  
176 grow older than in the field which caused an increase in wear of the teeth. The 2D outline of the  
177 occlusal surface is measured low on the crown, mitigating this issue (Renaud *et al*, 2017).

178 The UM1 outline was thus described using 64 points sampled at equal curvilinear distance using the  
179 Optimas software. The maximum length of the tooth was automatically extracted from this dataset.

180 The starting point was positioned at the most anterior part of the tooth.

181

### 182 *Morphometric analyses*

183 Molar size. – The relationship between molar length and body weight was tested using a linear  
184 regression on the subset of mice that were trapped on Orkney in 2012 and sacrificed at capture.  
185 Mice subsequently bred in captivity were not considered in this analysis. Differences in molar length  
186 between continental, Scottish and Orkney mice were tested using an analysis of variance (ANOVA)  
187 followed by pairwise Tukey post-hoc tests.

188 Procrustes superimposition. – The points along the outline were analysed as sliding semi-landmarks  
189 (Cucchi *et al*, 2013) using a generalized Procrustes superimposition (GPA) standardizing size, position  
190 and orientation while retaining the geometric relationships between specimens. During the  
191 superimposition, semi-landmarks were allowed to slide along their tangent vectors until their  
192 positions minimized the shape difference between specimens, the criterion being bending energy.

## Evolution of Orkney mice

193 Because the first point was only defined as a maximum of curvature, some slight offset might occur  
194 between specimens. It was therefore considered as a semi-landmark and allowed to slide between  
195 the last and second point. Two superimpositions were performed, one including the total dataset,  
196 and the other focusing on Orkney mice. Differences between groups were tested using Procrustes  
197 ANOVA (10000 permutations). The GPA and the Procrustes ANOVA were performed using the R  
198 package geomorph (Adams and Otárola-Castillo, 2013).

199 Multivariate analyses and statistics. – Principal component analyses (PCA) on the aligned coordinates  
200 were used to visualize the pattern of total variance and to reduce the dimensionality of the data, by  
201 retaining only PCs > 1% of variance in the subsequent analyses. Relationships between groups were  
202 further investigated using between-group PCA (bgPCA). While the PCA is an eigenanalysis of the total  
203 variance-covariance in the dataset, the bgPCA analyses the variance-covariance between group  
204 means, weighted by the sample size of each group. Relationships between groups were also  
205 visualized using unrooted neighbour-joining trees based on Euclidean distances between group  
206 means.

207

### 208 *Comparison between morphometric and genetic data*

209 The match between the genetic and morphometric structure was investigated at different scales and  
210 using different complementary approaches. (1) The relationship between the microsatellite and  
211 morphometric datasets was visualized using a co-inertia analysis. This approach aims to find  
212 orthogonal vectors (co-inertia axes) maximizing the covariance between the two datasets (Dolédéc  
213 and Chessel, 1994), allowing their projection in a common space. (2) The relationship between the  
214 microsatellite and morphometric datasets was tested using Protests (Peres-Neto and Jackson, 2001)  
215 and RV tests (Escoufier, 1973), the significance being based on 10000 permutations. These tests were  
216 performed on the complete datasets (all microsatellite data vs. aligned coordinates) and using a  
217 reduction of dimensionality, retaining only PC axes explaining more than 1% of variance in each case.

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218 For that purpose, the microsatellite dataset was analysed using a PCA. (3) The degree of phylogenetic  
219 signal in a morphometric dataset can be estimated by comparison with a reference phylogenetic tree  
220 (statistics  $K_{mult}$ ) (Adams, 2014). This approach compares the observed morphometric dataset to the  
221 expectation of evolution along the tree under a Brownian motion model, significance being assessed  
222 by permuting the shape data among the tips of the phylogeny. The significance was assessed based  
223 on 10000 permutations. Two reference phylogenies were used to address two different geographic  
224 scales. First, the neighbour-joining tree based on the D-loop p-distances was used to compare Orkney  
225 and continental European populations. The match between morphometric and genetic sampling was  
226 only possible at the level of the locality (or even neighbouring area) and group means had to be  
227 considered. We also used this phylogeny to test for differences in the net rates of morphological  
228 evolution between Orkney, Scotland and the continent. For this test, the net rate of shape evolution  
229 for each group in the multi-dimensional space is calculated under a Brownian motion model of  
230 evolution, and a ratio of rates is obtained (Adams, 2014). Second, the neighbour-joining tree based  
231 on the microsatellite distances was used to investigate the morphological diversification within  
232 Orkney. For this dataset, the match between genetic and morphometric variation was assessed at  
233 the individual level.

234 The PCA, bgPCA, co-inertia analyses and the RV tests were performed using the R package *ade4* (Dray  
235 and Dufour, 2007). Protests were performed using the R package *vegan* (Oksanen *et al*, 2013).  $K_{mult}$   
236 tests were performed using the R package *geomorph* (Adams and Otárola-Castillo, 2013).

237

238 **Results**239 *Continental vs Orkney mice: phylogeny and morphometrics*

240 *D-loop based phylogeny.* – In the phylogenetic tree reconstructed with the 728 haplotypes (Fig. S2),  
241 most of the sequences from Orkney belong to the well supported (PP = 0.9) and previously defined  
242 “Orkney lineage” or clade F (Jones *et al*, 2011b; Searle *et al*, 2009). The two other sequences are in

## Evolution of Orkney mice

243 the clade E (Jones *et al*, 2011b) or at the base of the phylogeny.

244 Molar size differences between populations. – Molar length was not related to body weight in the  
245 subset of 75 mice sacrificed at capture on Mainland, Papa Westray and South Ronaldsay ( $P = 0.837$ ).  
246 Orkney, continental European and Scottish mice differed in molar length (ANOVA:  $P < 0.0001$ ).  
247 Orkney mice had longer molars (average length  $\pm$  standard deviation:  $1.82 \pm 0.09$  mm) than their  
248 continental relatives ( $1.77 \pm 0.08$  mm) (Tukey:  $P < 0.0001$ ). Scottish mice had molars of  
249 intermediate length ( $1.79 \pm 0.08$  mm) and were therefore different neither from the continental  
250 ones ( $P = 0.4081$ ) nor the Orkney ones ( $P = 0.3656$ ). The range of within-population variation was  
251 large (Fig. 1A). Populations with long molars occurred both on the continent (Denmark [DK] and  
252 Brittany [TKH]) and Orkney (Eday, Faray, and Papa Westray).

253

254 Molar shape differentiation between the continent and Orkney. – Molar shape differentiation  
255 between populations from the continent, Scotland and Orkney was highly significant, (Procrustes  
256 ANOVA:  $P = 0.0001$ ). The differentiation of molar shape between mice from Orkney and those from  
257 western continental Europe was expressed both along PC1 (30% of variance) and PC2 (28.3%) (Fig.  
258 1B). Molars from Orkney mice were as widely distributed in the morphospace as those of continental  
259 mice, with parallel variation in Orkney and the continent mostly expressed along PC1. This axis  
260 described an elongation of the anterior part of the UM1. Tooth shape variations along PC2 were  
261 more localized, and mostly involved the labial anterior cusp. Molars from Scottish mainland mice  
262 were mostly within the range of molars from Orkney mice.

263 Considering a between-group PCA (bgPCA), the difference between localities corresponded to 32.3%  
264 of the total variance (Fig. 2A). The first axis (53.4%) differentiated continental from Scottish localities,  
265 including those in Orkney. The diversification within continental Europe and within Orkney was  
266 expressed on bgPC2 (26.1%). The two groups from Papa Westray (1992 and 2012) closely resembled  
267 each other. Two localities departed from the pattern separating Orkney from continental

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268 populations: Denmark [DK] grouped with Orkney, while the South Ronaldsay grouped with  
 269 continental populations (Fig. 2A, 2B).

270 The between-group morphometric distance matrix was only weakly correlated ( $R = 0.2251$ ,  $P =$   
 271  $0.0256$ ) to the D-loop p-distance matrix. A reduced multivariate morphometric dataset (first ten PCs  
 272 totalling 93% of variance) was compared to the D-loop phylogenetic tree (Fig. 2C), showing that  
 273 tooth shape did not evolve according to Brownian motion ( $K_{mult} = 0.00716$ ,  $P = 0.7418$ ).

274 Differences of evolutionary rates between groups were tested further. The difference between  
 275 continental Europe + mainland Scotland vs Orkney was not significant ( $P = 0.2178$ ), despite very  
 276 different average evolutionary rates (continental Europe + mainland Scotland = 0.022 vs Orkney =  
 277 3.889), possibly because of high heterogeneity within groups. Considering four groups (Orkney  
 278 Mainland, small Orkney islands, mainland Scotland, and the continent), the difference in evolutionary  
 279 rates was significant ( $P = 0.0465$ ; evolutionary rates for continental Europe = 0.025, mainland  
 280 Scotland = 0.011, Orkney Mainland = 3.727, small Orkney islands = 4.051).

281

282 *Genetic structure in Orkney*

283 Population structure based on the D-loop. – Seventeen haplotypes were distinguished among the 146  
 284 D-loop sequences from the Orkney archipelago. Fifteen belonged to the “Orkney lineage” (Fig. S2)  
 285 and among them, 11 were restricted to only one of the Orkney islands (Fig. 3A; Table 1).  
 286 In the network (Fig. 3B), 14 haplotypes are grouped together around a central haplotype (hap\_6),  
 287 which is present in several localities on Mainland and on three other Orkney islands. The remaining  
 288 two haplotypes are only distantly related to each other and to the other Orkney haplotypes. All the  
 289 haplotypes in the “Orkney lineage” are only separated by one or two mutational steps. Most of them  
 290 are restricted to one or a few localities. Therefore, each island, and each group identified in  
 291 Mainland, is characterized by its own haplotype composition.

292 Microsatellite analyses. – The 19 loci showed high levels of polymorphism with 5-18 (average 10.32)

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293 alleles per locus, and a total of 196 alleles across all sampling locations (Table 1). The different islands  
294 displayed low to moderate levels of observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity ranging from  
295 0.23–0.47 for the former and from 0.21–0.61 for the latter (Table 1).  $F_{st}$  values between islands were  
296 high ( $F_{st} = 0.21-0.82$ , Table S3) and highly significant ( $P < 0.001$ ). The populations on Mainland were  
297 genetically structured, as indicated by high  $F_{st}$  values (mean = 0.27; Table S4). The comparisons were  
298 significant ( $P < 0.01$ ) in most cases, except for localities sampled by a single specimen (Table S4).  
299 The DAPC analyses demonstrated that the populations were highly structured at the scale of the  
300 Orkney archipelago (Fig. 4A, B; 21 clusters) and at the scale of Mainland alone (Fig. 4C, D; 13  
301 clusters). Within Orkney, the different islands clearly separated from each other on the plane defined  
302 by the first two axes of the DAPC (Fig. 4A). The first axis separates Faray, Westray and to a lesser  
303 extent, Eday from the other islands. The Mainland, South Ronaldsay, Eday, Sanday and Papa Westray  
304 populations are spread along the second axis, each being well separated from the other islands. The  
305 Papa Westray population did not change over time. A focus on Mainland (Fig. 4C, D) revealed a  
306 strong geographic structure very similar to the one that was found with the D-loop (Fig. 3A),  
307 supporting the grouping of populations into differentiated regions. The only notable discrepancy  
308 concerns mice from the localities in the central part of Mainland (A5, A7). They harboured a D-loop  
309 haplotype that did not belong to the “Orkney lineage”, suggesting an import from elsewhere.  
310 However, the mice from these localities group with those from other Mainland localities with respect  
311 to the microsatellite data, showing no evidence of an allochthonous genetic signature.  
312 Finally, isolation by distance was stronger within the Orkney archipelago ( $R^2 = 0.135$ ,  $P < 0.001$ ) than  
313 within Mainland ( $R^2 = 0.023$ ,  $P = 0.03$ ).

314

315 *Relationship between microsatellite and morphometric data*

316 Orkney archipelago. – Morphometric and microsatellite data were available for 268 specimens from  
317 Orkney. Molar shape differences were significant between populations (Procrustes ANOVA:  $P =$

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318 0.0001) and explained 25.8% of the total variance, based on the between-group PCA. The first bgPCA  
319 axis tended to separate small islands from Mainland (Fig. 5A). Papa Westray samples from 1992 and  
320 2012 were close to each other and diverged along bgPC2, due to an anterior elongation of the tooth.  
321 Mainland groups were relatively close to each other.

322 The congruence between microsatellite and morphometric data was visualized using a co-inertia  
323 analysis (Fig. 5B). The overall match was good (short arrows), with populations from the small islands  
324 tending to differentiate less in tooth shape than expected based on the microsatellites (arrows  
325 pointing to each other, as exemplified for Faray, Westray, Sanday and Papa Westray). Mainland  
326 populations NC and DE tended to differentiate more in tooth shape than for the microsatellites  
327 (diverging arrows).

328 Microsatellites and morphometric datasets were compared based on between-group (N = 14) and  
329 individual (N = 268) estimates, using several tests (Protest, RV and Kmult) and using all variables and  
330 reduced datasets. The results were congruent overall (Table S5; only results based on Kmult test on  
331 the total datasets, i.e. 196 variables for the microsatellites and 128 aligned coordinates for the  
332 morphometric analysis, are provided in the text). Genetic and morphometric datasets appeared  
333 significantly correlated (all specimens, PKmult = 0.0003).

334 Local scale on Mainland. – There was a significant difference in tooth shape between the regions of  
335 Orkney Mainland, as determined from the microsatellite analysis (N = 174, Procrustes ANOVA: P =  
336 0.0001). Microsatellite and morphometric structures were significantly related (PKmult = 0.0001).  
337 Lastly, the analysis was focused on two sets of neighbouring farms. Northeast Mainland and  
338 Southeast Mainland were selected because of the good sample sizes and high genetic homogeneity  
339 at these locations.

340 For Northeast Mainland (N = 56), molar shape differed between neighbouring farms (Procrustes  
341 ANOVA: P = 0.0122) and the morphometric differentiation was related to the phylogenetic signal  
342 (PKmult = 0.0468).

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343 The pattern was similar in Southeast Mainland (N = 50). Molar shape differed between neighbouring  
344 populations (Procrustes ANOVA:  $P = 0.0231$ ), and morphometric divergence followed a Brownian  
345 motion along the phylogeny (PKmult = 0.0020).

346

347 **Discussion**348 *A low genetic diversity in a remote northern archipelago*

349 The present data confirm the dominance of the “Orkney lineage” (Searle *et al*, 2009) on the  
350 archipelago, attributed to an introduction by the Vikings (Searle *et al*, 2009). Twelve of the 15 D-loop  
351 haplotypes belonging to this clade are endemic to the Orkney archipelago. The three haplotypes  
352 found outside Orkney probably represent ancestral diversity, whereas the endemic ones may be the  
353 result of local differentiation (Searle *et al*, 2009). This dominance of a single haplogroup can be  
354 interpreted as the consequence of the first invading population being resilient to subsequent  
355 invasion, as documented in Kerguelen (Hardouin *et al*, 2010) and Madeira (Günduz *et al*, 2001). In  
356 contrast, a high genetic diversity was found in more meridional locations such as the Canary islands,  
357 Aeolian archipelago and the Azores (Bonhomme *et al*, 2011; Gabriel *et al*, 2015; Solano *et al*, 2013);  
358 Cyprus displays the highest mitochondrial diversity of any island population, with nine of the eleven  
359 described haplogroups present (García-Rodríguez *et al*, 2018).

360 The contrast between high genetic diversity in meridional islands and moderate to low diversity in  
361 Orkney and other high latitude islands is also evident from microsatellite data (Gabriel *et al*, 2013;  
362 Hardouin *et al*, 2010; Jones *et al*, 2011a; Jones *et al*, 2012); Cyprus here again displays the highest  
363 diversity (García-Rodríguez *et al*, 2018).

364 The genetic diversity in meridional islands reflects multiple colonization events which can be ascribed  
365 to their complex human history, especially in the case of the Mediterranean islands such as Cyprus,  
366 which was colonized as early as the Neolithic and experienced a considerable volume of sea traffic  
367 over several millennia (Cucchi *et al*, 2020; Cucchi *et al*, 2005; García-Rodríguez *et al*, 2018; Solano *et*

## Evolution of Orkney mice

368 *al*, 2013). In contrast, Orkney has been relatively isolated since the Viking period, as confirmed by  
369 human genetics, with the human population of Orkney differing substantially from other British  
370 populations and including an important contribution from Norway in its ancestry (Leslie *et al*, 2015).  
371 The resilience of house mouse populations to later invasion is not complete, however, as shown by  
372 two haplotypes (six mice) belonging to other clades that are mostly found in localities of mainland  
373 Britain (Searle *et al*, 2009), tracing more recent exchanges between Britain and Orkney. Assimilation  
374 nevertheless occurred, since these mice display a typical Orkney microsatellite signature.

375

376 *Orkney mice: an initial adaptive step?*

377 Despite the morphological diversity among the islands of the archipelago [(Ledevin *et al*, 2016), this  
378 study], the first order morphological signal is a divergence of all Orkney mice from continental ones.  
379 This idiosyncratic Orkney molar shape echoes what has been found for mandible shapes (Souquet *et*  
380 *al.*, 2019). This result was expected given the genetic homogeneity of Orkney mice, and yet, molar  
381 shape evolution appeared to be weakly related to neutral genetic evolution. This relative decoupling  
382 may be due to accelerated evolution on Scottish mainland and Orkney, an effect which likely  
383 increases in smaller and presumably, more isolated islands. A component of adaptation to northern  
384 environment may further contribute to the divergence of Orkney mice, since mice from Northern  
385 Scotland and Denmark present morphological similarities with Orkney mice, despite their different  
386 haplotypic signatures and translocation history (Searle *et al.*, 2009).

387 Beyond shape differentiation, an increase in body size is expected for insular small mammals  
388 (Lomolino, 1985; Lomolino, 2005). Body size response was difficult to assess here because most of  
389 the Orkney mice were kept for a while in laboratory conditions, allowing them to grow older and  
390 larger. Molar size is considered to be a good proxy of body size at a broad taxonomic scale (Gingerich  
391 *et al*, 1982), but not at a population level, because the first molar erupts early after birth and is  
392 therefore not affected by subsequent growth (Renaud *et al.*, 2017). As a consequence, the increase

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393 in molar size observed on some Orkney islands is probably not related to differences in body size,  
394 and the larger molar size of Orkney mice may not be indicative of their larger body size compared to  
395 mice from the continent.

396

397 *A strong genetic structure within the Orkney archipelago*

398 Beyond the typical Orkney signature, molar shape diversified within Orkney. The population  
399 structure based on mitochondrial and microsatellite data is highly congruent with this morphological  
400 diversification.  $F_{st}$  values are high for an intraspecific structure, but are similar to what has been  
401 observed for other insular house mouse populations such as those in the Azores, Madeira, and Faroe  
402 (Gabriel *et al.*, 2013; Jones *et al.*, 2011a). Divergence between populations from the different Orkney  
403 Islands was also found for Orkney voles (Cucchi *et al.*, 2014). The fragmentation of the archipelago  
404 obviously constitutes a barrier to human and animal exchanges between islands.

405 The population structure observed on Orkney Mainland is more intriguing, since no visible  
406 geographic barriers divide the landscape, except perhaps for the narrow isthmus connecting the  
407 Deerness peninsula. Accordingly, no genetic structure was observed for Mainland Orkney voles  
408 (Martínková *et al.*, 2013). House mice are supposed to be readily translocated by people, even at a  
409 local scale, to an extent that will erase any geographic structure on an island as large as Cyprus  
410 (García-Rodríguez *et al.*, 2018). However, on Mainland Orkney, they appear to have accumulated  
411 more geographic structure than voles, within a much shorter time span. This small-scale geographic  
412 structure may firstly be due to the cool and wet climate of Orkney, which may dissuade mice from  
413 foraging extensively outdoors, although Orkney mice could occasionally be trapped in fields during  
414 summer time. Secondly, house mice are at a competitive disadvantage to wood mice (*Apodemus*  
415 *sylvaticus*) beyond the vicinity of human buildings and activity (Berry and Tricker, 1969; Fairley and  
416 Smal, 1987). This could contribute to their restricted overland dispersal in Mainland, given that wood  
417 mice were introduced there several millennia before the arrival of house mice (e.g. (Romaniuk *et al.*,

## Evolution of Orkney mice

418 2016)). Indeed, competition with the wood mouse appeared to be an important factor influencing  
419 tooth shape diversification of house mice from the islands of Orkney (Ledevin et al., 2016).  
420 The congruence of the microsatellite signal with the more slowly evolving mitochondrial genetic  
421 variation underlines the long-lasting stability of this geographic structure. A dynamic of local  
422 extinction and recolonization is typical for synanthropic house mouse populations which are known  
423 to function in small demes, with groups of related individuals structured at a very small geographic  
424 scale of only a few metres (Pocock *et al*, 2004). In this process, human-mediated translocations can  
425 erase any geographic structure; however, our results suggest that there have been few such  
426 exchanges across Mainland. This may indeed be related to local human practices, since people in the  
427 north-eastern, north-western, and south-eastern parts of Mainland Orkney appear to be genetically  
428 differentiated (Gilbert *et al*, 2019).  
429 The congruence of human and house mouse phylogeography is well-known (Searle *et al*, 2009) and  
430 the house mouse has consequently been considered as a bio-proxy to infer past human long-distance  
431 travels (Jones *et al*, 2013). Our data suggest that the structure of house mouse populations may even  
432 reflect human spatial organization and social practices at a surprisingly small local level.

433

434 *Isolation and fragmentation as drivers of morphological diversification*

435 A strong and small-scale genetic structure therefore resulted from behavioural patterns of both mice  
436 and men. This genetic structure is tightly mirrored in tooth shape, which displays a high disparity  
437 among Orkney islands and even within Mainland. An analysis of the molar row topography, however,  
438 suggested that the most extreme Orkney phenotypes were not functionally advantageous and may  
439 even be the result of a relaxation of functional demands in the insular environment (Renaud *et al*,  
440 2018; Souquet *et al*, 2019).

441 The morphological evolution on Orkney thus appears to be largely neutral and to relate to isolation  
442 between populations. It is notable that isolation occurred even between neighbouring groups of

## Evolution of Orkney mice

443 farms, triggering differences in upper molar shape even at this very small geographic scale because  
444 of developmental properties favouring rapid evolution (Hayden *et al*, 2020).

445

### 446 **Conclusions**

447 On the continent, frequent translocations of mice, associated with local extinctions, are likely  
448 continually reshuffling genetic composition and consequently erasing local morphological  
449 divergence, resulting in a rather homogeneous molar tooth morphology (Ledevin *et al.*, 2016;  
450 Renaud *et al.*, 2017). The ability of house mice to rapidly evolve in small fragmented populations may  
451 fuel genetic and morphological diversity, providing ample variation for the action of selection, and  
452 possibly contributing to the success of the house mouse as a worldwide invasive species.

453 Nevertheless, while adaptation to local conditions might have initially contributed to the evolution of  
454 Orkney mice, our data suggest that drift is the primary driver sustaining morphological disparity in  
455 these fragmented and isolated populations. Whether or not this is the case, the genetic structure  
456 was clearly mirrored by the morphological evolution, demonstrating that molar shape is a useful  
457 marker of evolution at very short time scales.

458

### 459 **Acknowledgements**

460 We warmly thank Josette Catalan and Annie Orth for their participation to the 2012 field trip to  
461 Orkney, Jeremy Searle for original collection of specimens from northern Scotland, and all the people  
462 from Orkney who kindly made possible the extensive trapping campaigns. The manuscript benefited  
463 from the constructive remarks of three anonymous reviewers and Stuart J.E. Baird.

464 This work was performed using the computing facilities of the CC LBBE/PRABI. It was supported by  
465 the ANR Bigtooth (ANR-11-BSV7-008). This is a publication ISEM 2020-216.

466

### 467 **Conflict of Interest:**

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468 The authors declare no conflict of interest

469

470 **Data Archiving:**

471 - The DNA sequences are deposited in EMBL, accessions LR862585-LR862663.

472 - Sampling locations are indicated in Table S1.

473 - Morphological data and microsatellite genotypes are deposited in Dryad:

474 doi:10.5061/dryad.nvx0k6dqm

475

476 Supplementary information is available at Heredity's website.

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670

671

672

673 **Figure Legends**

674

675 **Figure 1.** A) Variation in tooth length among populations. B) Morphospace describing molar shape in  
676 continental (grey), Scottish (blue), and Orkney (green) mice. The first two axes of a PCA on the  
677 aligned coordinates are displayed. Tooth shape changes from the minimum to the maximum scores  
678 along PC1 and PC2 are depicted to the right.

679

680 **Figure 2.** Between-group tooth shape differentiation. A) Differentiation of the groups on the first two  
681 axes of a between-group PCA on the aligned coordinates. B) Neighbour-joining tree based on  
682 morphometric distances between the group means. C) Neighbour-joining tree based on average  
683 molecular p-distances calculated between the groups. Continental Western Europe in grey, Scotland  
684 in blue, Orkney in green.

685

686 **Figure 3.** A) Distribution of the 17 haplotypes sampled in Orkney. B) Network of the haplotypes  
687 present in Orkney. The colours correspond to the different haplotypes.

688

689 **Figure 4.** DAPC analysis of the microsatellite data at the level of the Orkney archipelago (A) with the  
690 projection of the clusters present on the small islands (B), and at the level of Mainland alone (C) with  
691 the projection of the 21 clusters present in the whole archipelago (D). In A and C, the number of the  
692 cluster resulting from the DAPC analysis is indicated at the centre of the corresponding group.

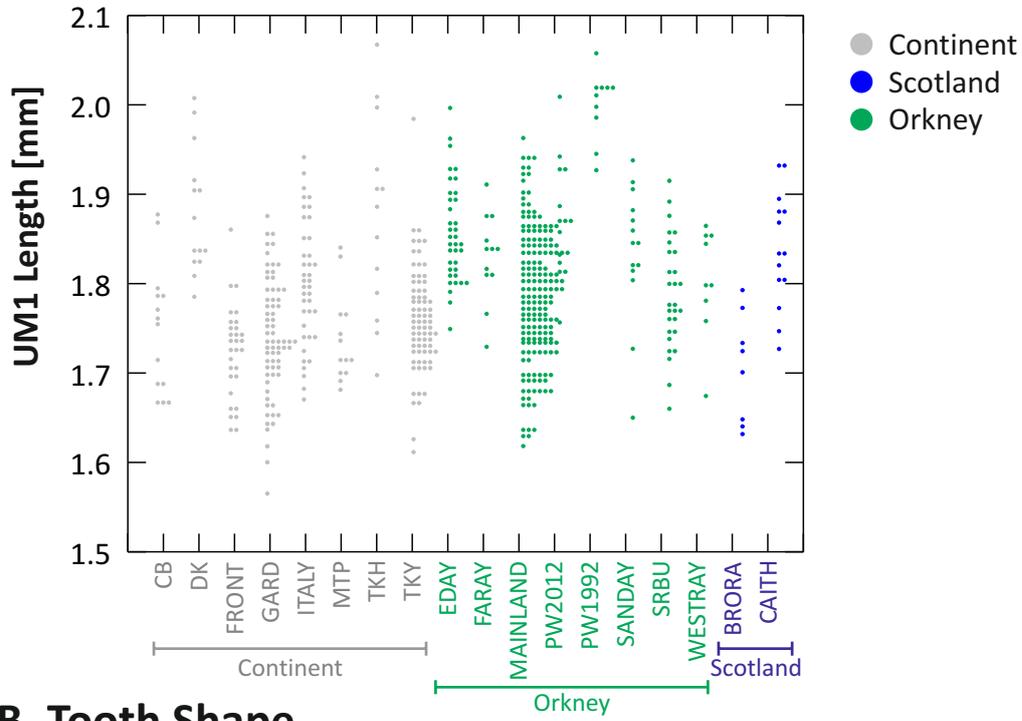
693

694 **Figure 5.** A) Molar shape differentiation on Orkney, based on a between-group PCA on the aligned  
695 coordinates of the molar outline. To the left, visualization of the shape changes along the axes. B)  
696 Coinertia analysis between microsatellite and morphometric datasets. The arrows indicate the  
697 change in topology going from microsatellites to morphometrics. Colour code as in Fig. 4D.

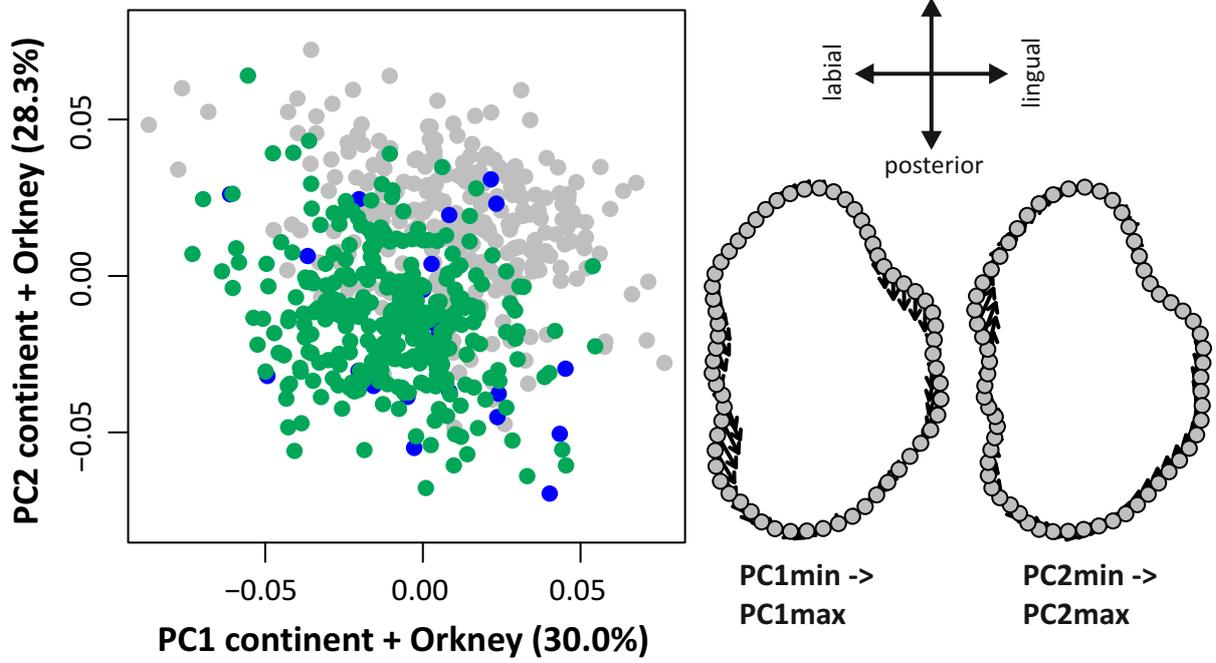
698 **Table Legend**

699 **Table 1.** Number of house mice trapped on Orkney islands and included in the different analyses,  
700 genetic diversity measures based on the D-loop and 19 microsatellite loci datasets, and island size.  
701

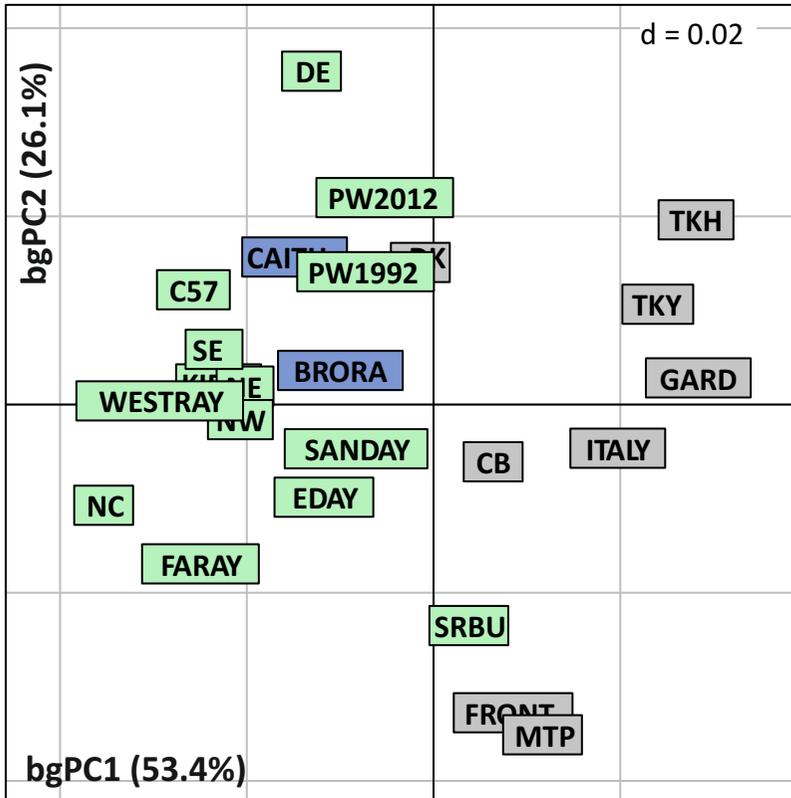
## A. Tooth Size



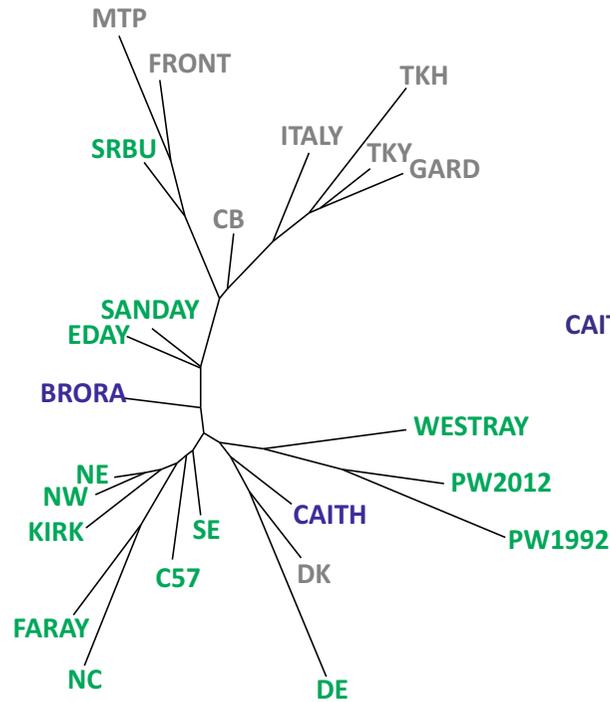
## B. Tooth Shape



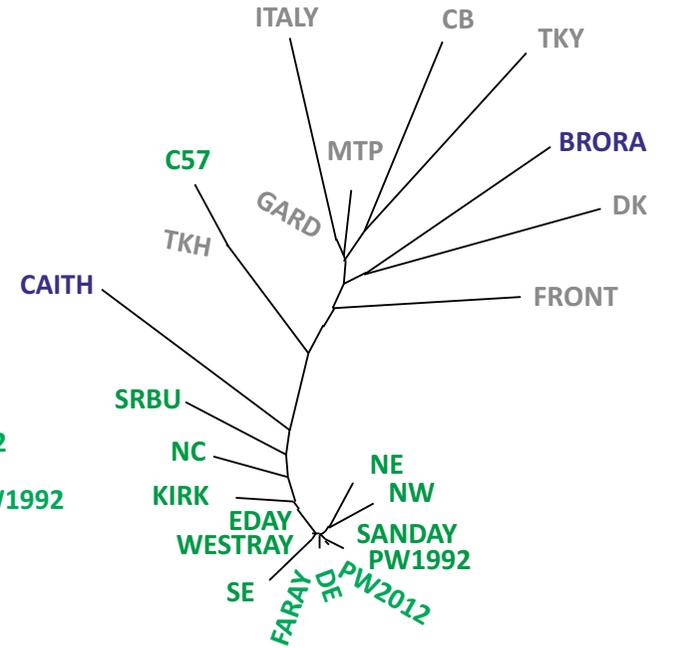
**A. Tooth shape morphospace**



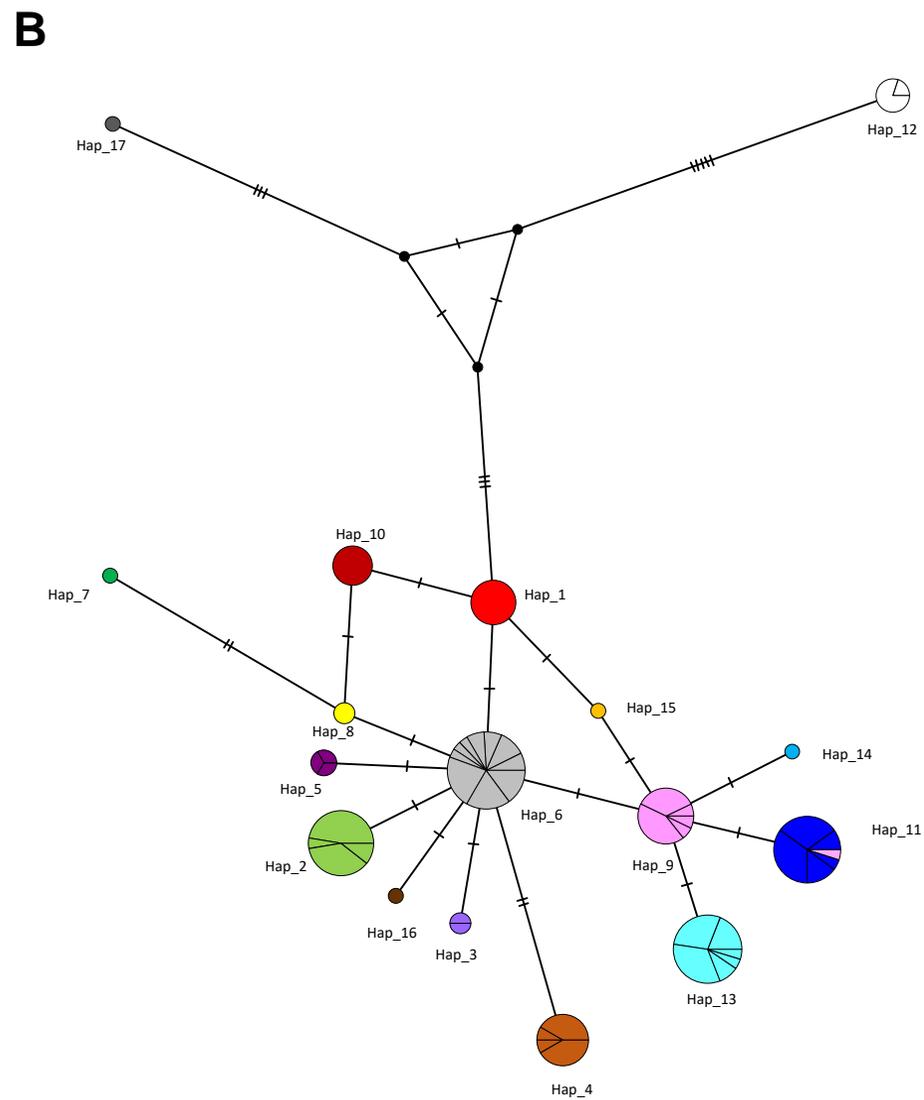
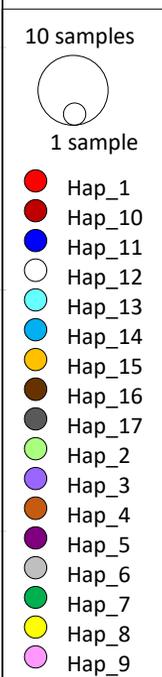
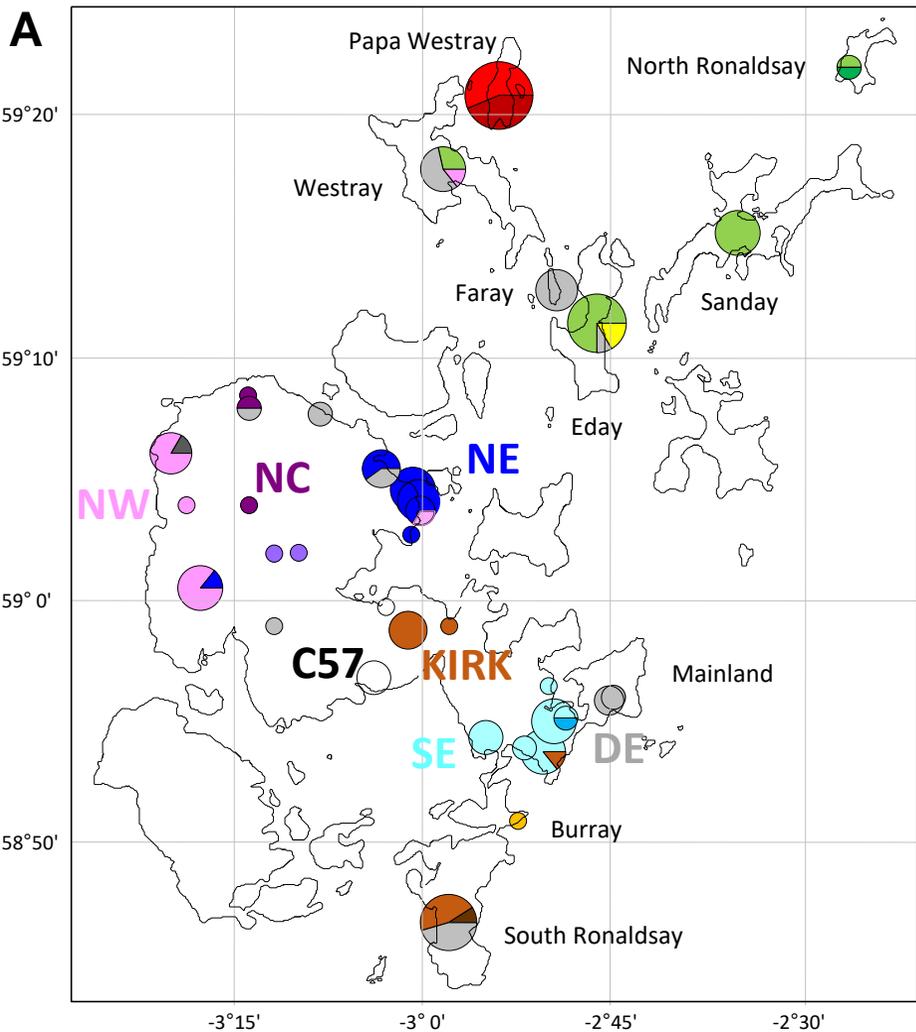
**B. Tooth shape NJ**

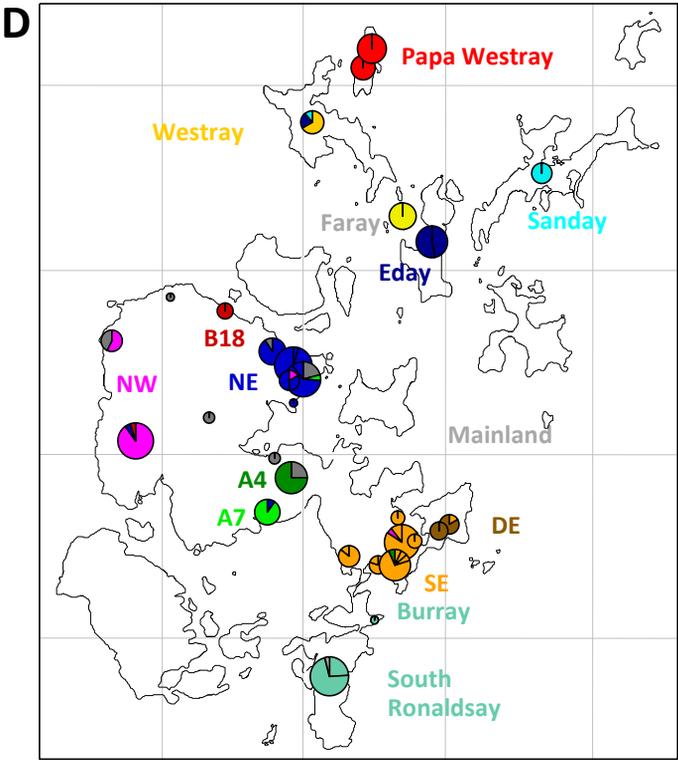
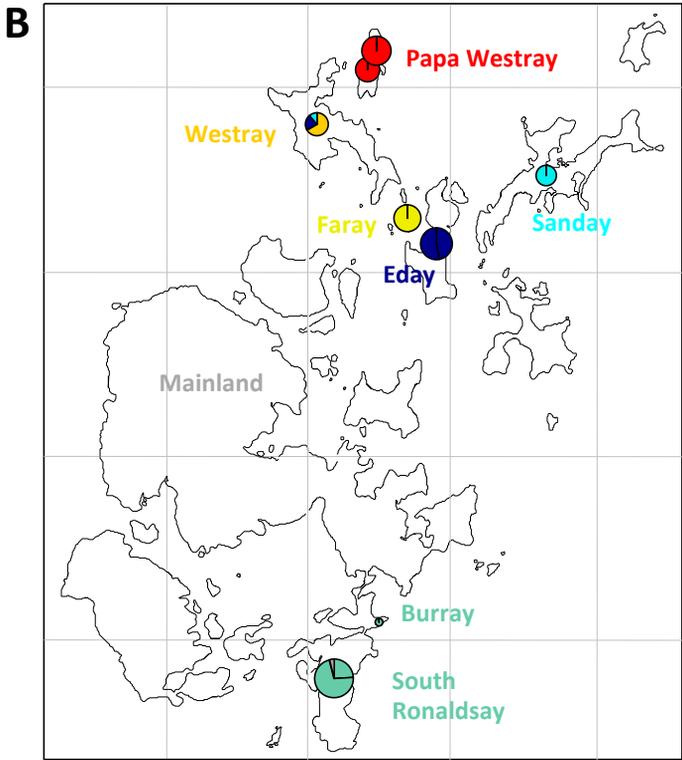
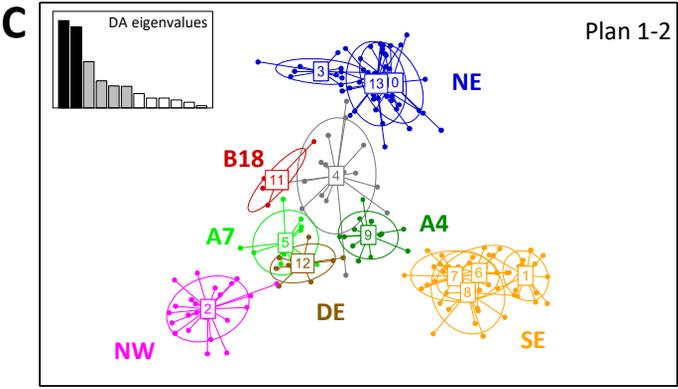
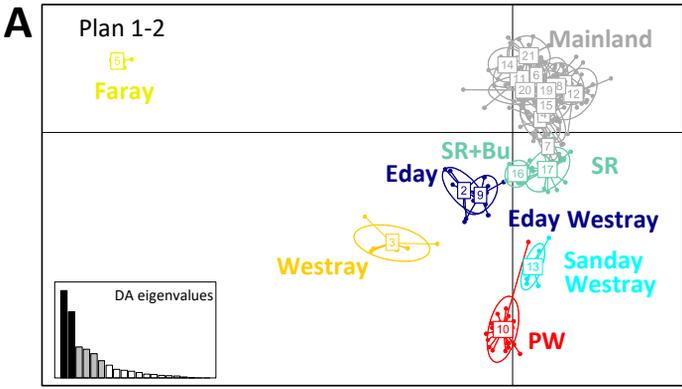


**C. D-loop NJ**



0.002







**Table 1.** Number of house mice trapped on Orkney islands and included in the different analyses, genetic diversity measures based on the D-loop and 19 microsatellite loci datasets, and island size.

	Area (km <sup>2</sup> )	NDloop	N <sub>H</sub>	h ± SD	π ± SD	Nmic	A (Range)	Ho	He	Nmor	Nmm
Orkney (all islands)	990	146	17	0.8913 ± 0.0091	0.004191 ± 0.002360	279	10.32 (5-18)	0.39	0.68	303	268
Mainland	523.25	84	10 (5)	0.8371 ± 0.0180	0.003505 ± 0.002040	184	7.89 (3-18)	0.43	0.61	179	174
South Ronaldsay	49.8	11	3 (1)	0.6364 ± 0.0895	0.001451 ± 0.001112	25	4.16 (1-7)	0.47	0.54	27	25
Burray	9.03	1	1 (1)	1	0	1	1.16 (1-2)			1	1
Eday	27.45	12	3 (1)	0.4394 ± 0.1581	0.002675 ± 0.001765	17	2.89 (1-8)	0.34	0.4	37	16
Faray	1.8	6	1 (0)	0	0	12	1.21 (1-2)	0.23	0.21	12	12
North Ronaldsay	6.9	2	2 (1)	1.0000 ± 0.5000	0.009101 ± 0.009653	--	--	--	--	--	--
Papa Westray	9.18	16	2 (2)	0.5250 ± 0.0546	0.000598 ± 0.000590	24	3 (1-6)	0.32	0.36	24	24
Sanday	50.43	7	1 (0)	0	0	7	2.21 (1-5)	0.42	0.48	14	7
Westray	47.13	7	3 (0)	0.6667 ± 0.1598	0.003041 ± 0.002104	9	4.26 (1-7)	0.37	0.61	9	9

NDloop: number of Dloop sequenced; k: mean number of pairwise difference; N<sub>H</sub>: number of haplotype, private haplotypes are indicated within brackets; h: haplotype diversity, π: nucleotide diversity; Nmic: number of mice scored at 19 microsatellites; A: mean number of alleles per locus with the range of alleles number in brackets; observed (Ho) and expected (He) heterozygosities; Nmor: morphometrics; Nmm: match microsatellites + morphometrics.