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2 **Evidence for an extreme founding effect in a highly successful invasive species**

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12

13 **Abstract**

14 The adaptive potential of invasive species is thought to decrease during founding events due to  
15 reduced genetic diversity, limiting the new population's ability to colonize novel habitats.  
16 Barbary ground squirrels (*Atlantoxerus getulus*) were purportedly introduced as a single breeding  
17 pair to the island of Fuerteventura but have expanded to over a million individuals spread across  
18 the island in just over 50 years. We estimated the number of founders and measured the level of  
19 genetic diversity in this population using the mitochondrial displacement loop and microsatellite  
20 markers. Island samples ( $n = 19$ ) showed no variation in the d-loop, suggesting a single founding  
21 female, while Moroccan samples ( $n = 6$ ) each had unique mitochondrial haplotypes. The  
22 microsatellite data of the island population ( $n = 256$  individuals) revealed a small effective  
23 population size, low levels of heterozygosity, and high levels of inbreeding, supporting a  
24 founding population size of two to three individuals. Our results suggest that *A. getulus* has  
25 undergone an intense genetic bottleneck during their colonization of the island. They are one of  
26 the few species where introduction effort does not explain invasion success, although further  
27 investigation may explain how they have avoided the worst expected effects following an  
28 extreme genetic bottleneck.

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30 **Keywords:** invasive species, inbreeding, effective population size, genetic bottleneck

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## 35 **1. Introduction**

36 Extreme population bottlenecks can produce inbreeding and subsequent inbreeding depression  
37 [1, 2] because genetic drift becomes more powerful than selection in small populations. When  
38 drift is strong, beneficial alleles can be lost and detrimental alleles fixed due to random chance.  
39 As homozygosity increases due to drift, phenotypes associated with deleterious alleles that are  
40 hidden in heterozygote states become exposed to selection, and inbreeding depression occurs [for  
41 reviews, see 3, 4]. The strength of drift is often not apparent from the census size of a population,  
42 as not all individuals contribute equally to the next generation and population size can recover  
43 from a bottleneck much faster than the population's genetic diversity. However, a population  
44 experiences drift at the rate of its effective population size, which underscores the fact that even  
45 large populations can continue to experience strong effects of drift and continued loss of genetic  
46 diversity [5,6].

47  
48 In some cases, the effects of inbreeding following extreme bottlenecks are not noticeable; thus,  
49 understanding the nature of such populations is important for conservation. Within invasive  
50 species ecology, many populations are paradoxically founded by a small number of individuals  
51 with reduced genetic diversity due to the small size of the available gene pool [7-9]. The ability  
52 of these species to adapt to and colonize novel environments can be jeopardized by low levels of  
53 genetic diversity [10]. But a sufficiently large founder population (number of individuals or  
54 genotypes) [e.g. 11, 12], or multiple introduction events, which introduce new alleles into the  
55 population [e.g. 13, 14; see also 7, 15] often characterize successful invasions. Bottlenecked  
56 populations that retain sufficient levels of variation may regain some genetic variability through  
57 mutation [10, 16, 17], increasing their likelihood of survival. Small founder populations without

58 subsequent introductions should, therefore, have decreased fitness and face difficulty when  
59 attempting to establish in novel environments. No successful establishment of an invasive  
60 mammal from either one breeding pair or one pregnant female has been recorded; an invasive  
61 population founded by either scenario would be an ideal study model for the founder effect [18].

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63 Here we quantify genetic diversity and estimate the effective population size of the invasive  
64 population of Barbary ground squirrels (*Atlantoxerus getulus*) on the island of Fuerteventura,  
65 Spain. Purportedly introduced as a breeding pair from Sidi Ifni, Morocco in 1965 [19], the  
66 current island population has had remarkable success in population growth (estimated one  
67 million) and range expansion [20, 21]. We examined the mitochondrial and nuclear diversity of  
68 *A. getulus* to resolve any discrepancies between the two differently inherited genomes [22-24].  
69 We targeted the mitochondrial displacement loop and nuclear microsatellites, as any variation in  
70 this recently established population would likely be found in the most rapidly evolving areas of  
71 the two genomes [25]. We expected to find a single mitochondrial haplotype, high levels of  
72 inbreeding, one to four microsatellite alleles at each nuclear locus, and a small effective  
73 population size on the island due to the exclusive presence of alleles from a single founding pair.  
74 With this research, we intend to contribute to the general knowledge on the role of genetic  
75 diversity and bottlenecks in explaining the success of biological populations.

76

## 77 **2. Methods**

### 78 (a) Study species, trapping locations and methods

79 We trapped *A. getulus* according to previously described methods in various locations on  
80 Fuerteventura and Morocco [see 26-29] and stored tissue samples in 95% ethanol. Mitochondrial

81 d-loop sequences were obtained from 45 animals, and 256 animals were genotyped at eleven  
82 microsatellite loci (see S.I. for details).  
83 We tested for inbreeding and variation from Hardy-Weinberg equilibrium using the “adegenet”  
84 package v.2.1.1 [30, 31] and the “pegas” package x.0.11 Monte Carlo exact test with 1000  
85 replicates [32], respectively, in R v.3.5.1 [33]. Alleles were determined to have been introduced  
86 by founders rather than mutation (i.e. “founding alleles”) if they had a frequency  $> 0.05$  and were  
87 more than one repeat unit away from a common allele [12]. We performed a principal  
88 component analysis (PCA) using the “ade4” package v.1.7-13 [34] to determine whether there  
89 was any genetic structure in the population. We then calculated effective population size ( $N_e$ )  
90 using the LDNE method, assuming random mating and setting the minor allele frequency to 0.05  
91 [35].

92

### 93 **3. Results**

#### 94 (a) Mitochondrial DNA

95 We found no variation among island squirrels, whereas all six individuals from Morocco had  
96 unique haplotypes and showed 16 variable nucleotide sites compared to island samples, despite  
97 the limited sample size of the Moroccan source (Fig. 1, Table 1). We found four variable sites  
98 (0.389%) between Fuerteventura sequences and M10, the Moroccan sequence most similar to  
99 those on the island (Fig. 1).

#### 100 (b) Nuclear DNA

101 We found no evidence of large allele dropout or scoring error due to stuttering [36]. Null alleles,  
102 indicated by homozygote excess, were present at five loci that were removed from the analysis  
103 [37, 38]. All remaining loci were in HWE ( $p > 0.05$ ). Each locus had between two and nine

104 alleles ( $4.36 \pm 2.11$ , mean  $\pm$  SD), the number of founding alleles ranging from one to five ( $2.73 \pm$   
105  $0.65$ ). Mean observed heterozygosity ( $H_O = 0.57$ ) was greater than expected ( $H_E = 0.55$ ; Table 1  
106 supplemental information) and the average level of inbreeding was high (Fig. 2: average  $F =$   
107  $0.23$  [ $0.10 - 0.60$ , min - max]). Since we found no evidence of population structure (S.I. Fig. 1),  
108 we assumed that our sample was representative of the entire island population. We estimated  $N_e$   
109 to be  $77.2$  (95% CI:  $56.3, 109.5$ ).

110

#### 111 **4. Discussion**

112 We characterized segments of the mitochondrial and nuclear genomes of a highly successful  
113 invasive island population of *A. getulus* to determine its genetic diversity and number of  
114 founders. We observed low genetic diversity, evidence of inbreeding in mitochondrial and  
115 nuclear DNA, and a single mitochondrial haplotype suggesting the presence of only one  
116 founding female. We found variation between each mitochondrial d-loop sequence of Moroccan  
117 samples despite a small sample size ( $n = 6$ ), whereas the island population did not show variation  
118 with a larger sample size ( $n = 19$ ).

119 Microsatellite data also supported the hypothesis that this island population was founded by a  
120 small number of individuals but data from marker Aget19 suggest that there may be more than  
121 two founders (S. I. Table 2). Of nine alleles at this locus, five are present at a frequency greater  
122 than 5% [12], which is incongruous with the hypothesis that the island population was founded  
123 by two individuals. However, two of these alleles (repeat lengths 319 and 339) have frequencies  
124 just above the threshold of being counted as true founder alleles ( $0.0573$  and  $0.0553$ ,  
125 respectively; S. I. Table 2). It is possible these alleles were introduced by a founder, but the  
126 potential that 319 and 339 are due to rare double mutations, genotyping error, or an early

127 mutation that was propagated over the threshold cannot be overlooked. Another microsatellite  
128 marker, Aget1, also has a high number of alleles but only two are present at high frequency (>  
129 5%). An interesting allele at this marker is repeat length 152, which is two repeat units away  
130 from a founder allele and therefore does not comply with the recommended criteria [12].  
131 However, it is present at low frequency (0.0108), and multiple mutations in the same location,  
132 while unlikely, are not impossible [12]. Further investigation may confirm the true origin of  
133 these alleles.

134 With an average inbreeding coefficient  $F$  of 0.23, the *A. getulus* population should be at a  
135 survival or range expansion disadvantage [39, 40], as an increased probability of extinction  
136 exists when  $F$  values are at or just below “intermediate” levels (0.30 – 0.40; 11, 41]. However,  
137 the species has successfully established and spread across the island [20, 21] in a genetic paradox  
138 of invasion [9]. *Atlantoxerus getulus* invasion success may be due to extrinsic habitat factors [22,  
139 29], or other species-level [42, 43], behaviour [28], or life-history traits [44]. Alternatively,  
140 inbreeding may have benefitted the population by purging deleterious founding alleles [41, 45].  
141 Despite an estimated population size of one million, the effective population size was  
142 approximately 77 individuals (0.0077%), which is very low compared to other infamously  
143 bottlenecked mammals. Northern elephant seals survived near extinction and experienced steady  
144 population growth from about 100 to over 200,000 individuals, with an  $N_e$  of approximately  
145 40,000 (>20%) [12, 46, 47]. Cheetahs are estimated to number 6674 individuals with an  $N_e$  of  
146 between 1001–2937 (15–44%) [48, 49]. Some re-introduced populations of European bison  
147 (*Bison bonasus*) have  $N_e/N$  values as low as 0.05 (5%) [50]. The island population of *A. getulus*,  
148 therefore, has one of the smallest recorded effective population sizes relative to their census size.

149 One caveat of our study was the sampling regime. The sampling density for mtDNA was low  
150 although samples were collected from sites across the entire island of Fuerteventura, whereas  
151 sampling density for nuclear DNA was higher but restricted to a single area. As such, we found  
152 no evidence of population structure. However, there are no geographic barriers to dispersal  
153 across the island, as squirrels have been observed in all regions [20, 21], thus population  
154 structure may be absent altogether. Better coverage of the island or perhaps the collection of  
155 whole genomes may provide further insight into this recent founding event.

156 We have shown that the *A. getulus* population on Fuerteventura has undergone an intense genetic  
157 bottleneck during their colonization of the island. However, despite their lack of genetic diversity  
158 and low effective population size, they have successfully established and spread across the  
159 island, providing an ideal example of the founder effect.

160

161 **Ethics.** All sample collection followed the animal care protocols of the University of Manitoba  
162 (Animal Care and Use Committee #F14-032) and the government of Fuerteventura (Cabildo  
163 Insular de Fuerteventura #14885). Samples from Morocco were obtained with the permission of  
164 the Ministry of Territory Development, Water and Environment of Morocco (512/0170 March  
165 2006) and brought back to the EU under the authorization of the Government of the Canary  
166 Islands.

167 **Data accessibility.** Data will be available at Dryad

168 **Authors' contributions.** JMW and ML-D discussed the idea and KVK, JMW and SDP designed  
169 the study. KVK, AVDM, ML-D and JMW conducted the field work. KVK and AVDM  
170 performed the lab work. KVK, CG, and SDP contributed to the analysis and interpretation of the  
171 data. All authors contributed to the manuscript preparation and revisions. All authors approved



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## 184 **References**

- 185  
186 1. Lande R. 1988 Genetics and demography in biological conservation. *Science* **16**, 1455-1460.  
187 (doi:10.1126/science.3420403)
- 188 2. Bijlsma R, Bundgaard J, Boerema AC. 2000 Does inbreeding affect the extinction risk of  
189 small populations?: predictions from *Drosophila*. *J. Evolution. Biol.* **13**, 502-514.  
190 (doi:10.1046/j.1420-9101.2000.00177.x)
- 191 3. Charlesworth D, Willis JH. 2009 The genetics of inbreeding depression. *Nat. Rev. Genet.* **10**,  
192 783-796. (doi:10.1038/nrg2664)
- 193 4. Ouborg NJ, Pertoldi C, Loeschke V, Bijlsma RK, Hedrick PW. 2010 Conservation genetics  
194 in transition to conservation genomics. *Trends Genet.* **26**, 177-187.  
195 (doi:10.1016/j.tig.2010.01.001)

- 196 5. Wright S. 1931 Evolution in Mendelian populations. *Genetics* **16**, 97-159. (PMID:17246615)
- 197 6. Charlesworth B. 2009 Effective population size and patterns of molecular evolution and  
198 variation. *Nat. Rev. Genet.* **10**, 195-205. (doi:10.1038/nrg2526)
- 199 7. Dlugosch KM, Parker IM. 2008 Founding events in species invasions: genetic variation,  
200 adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* **17**: 431-449.  
201 (doi:10.1111/j.1365-294X.2007.03538.x)
- 202 8. Simberloff D. 2009 The role of propagule pressure in biological invasions. *Annu. Rev. Ecol.*  
203 *Evol. S.* **40**, 81-102. (doi:10.1146/annurev.ecolsys.110308.120304)
- 204 9. Estoup A, Ravigné V, Hufbauer R, Vitalis R, Gautier M, Facon B. 2016 Is there a genetic  
205 paradox of biological invasion?. *Annu. Rev. Ecol. Evol. Syst.* **47**, 51-72.  
206 (doi:10.1146/annurev-ecolsys-121415-032116)
- 207 10. Labonne J, Kaeuffer R, Gueraud F, Zhou M, Manicki A, Hendry AP. 2016 From the bare  
208 minimum: genetics and selection in populations founded by only a few parents. *Evol. Ecol.*  
209 *Res.* **17**, 21-34.
- 210 11. Signorile AL, Wang J, Lurz PWW, Bertolino S, Carbone C, Reuman DC. 2014 Do founder  
211 size, genetic diversity and structure influence rates of expansion of North American grey  
212 squirrels in Europe?. *Divers. Distrib.* **20**, 918-930. (doi:10.1111/ddi.12222)
- 213 12. Abadía-Cardoso A, Freimer NB, Deiner K, Garza JC. 2017 Molecular population genetics of  
214 the northern elephant seal *Mirounga angustirostris*. *J. Hered.* **108**, 618-627.  
215 (doi:10.1093/jhered/esx053)
- 216 13. Barson NJ, Cable J, van Oosterhout C. 2009 Population genetic analysis of microsatellite  
217 variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic

- 218 source-sink metapopulation structure, founder events and population bottlenecks. *J.*  
219 *Evolution. Biol.* **22**, 485-497. (doi:10.1111/j.1420-9101.2008.01675.x)
- 220 14. Russell JC, Hobins JH, Fewster RM. 2019 Phylogeography of invasive rats in New Zealand.  
221 *Front. Ecol. Evol.* **7**, 48. (doi:10.3389/fevo.2019.00048)
- 222 15. Kolar CS, Lodge DM. 2001 Progress in invasion biology: predicting invaders. *Trends Ecol.*  
223 *Evol.* **16**, 199-204. (doi:10.1016/S0169-5347(01)02101-2)
- 224 16. Huchon D, Delsuc F, Catzeflis FM, Douzery EJP. 2002 Armadillos exhibit less genetic  
225 polymorphism in North American than in South America: nuclear and mitochondrial data  
226 confirm a founder effect in *Dasyus novemcinctus* (Xenarthra). *Mol. Ecol.* **8**, 1743-1748.  
227 (doi:10.1046/j.1365-294x.1999.00768.x)
- 228 17. Gloag R, Ding G, Christie JR, Buchmann G, Beekman M, Oldroyd BP. 2016 An invasive  
229 social insect overcomes genetic load at the sex locus. *Nat. Ecol. Evol.* **1**, 11.  
230 (doi:10.1038/s41559-016-0011)
- 231 18. Carson HL, Templeton AR. 1984 Genetic revolution in relation to speciation phenomena: the  
232 founding of new populations. *Ann. Rev. Ecol. Evol. S.* **15**, 97-131.  
233 (doi:10.1146/annurev.es.15.110184.000525)
- 234 19. Machado, A. 1979. The introduction of the Getulian squirrel (*Atlantoxerus getulus* L., 1758)  
235 in Fuerteventura, Canary Islands. *Egypt. J Wild. Nat. Res.* **2**, 182 – 202.
- 236 20. López-Darias M, Lobo JM. 2008 Factors affecting invasive species abundance: The Barbary  
237 ground squirrel on Fuerteventura Island, Spain. *Zool. Stud.* **47**, 268-281.
- 238 21. López-Darias M, Lobo JM, Gouat P. 2008 Predicting potential distributions of invasive  
239 species: the exotic Barbary ground squirrel in the Canarian archipelago and the west  
240 Mediterranean region. *Biol. Invas.* **10**, 1027-1040. (doi:10.1007/s10530-007-9181-2)

- 241 22. Haavie J, Sætre G-P, Moum T. 2000 Discrepancies in population differentiation at  
242 microsatellites, mitochondrial DNA and plumage colour in the pied flycatcher – inferring  
243 evolutionary processes. *Mol. Ecol.* **9**, 1137-1148. (doi:10.1046/j.1365-294x.2000.00988.x)
- 244 23. Fisher-Reid MC, Wiens JJ. 2011 What are the consequences of combining nuclear and  
245 mitochondrial data for phylogenetic analysis? Lessons from *Plethodon* salamanders and 13  
246 other vertebrate clades. *BMC Evol. Biol.* **11**, 300. (doi:10.1186/1471-2148-11-300)
- 247 24. Schneider TC, Kappeler PM, Pozzi L. 2016 Genetic population structure and relatedness in  
248 the narrow-striped mongoose (*Mungotictis decemlineata*), a social Malagasy carnivore with  
249 sexual segregation. *Ecol. Evol.* **6**, 3734-3749. (doi:10.1002/ece3.2123)
- 250 25. Wan Q, Wu H, Fujihara T, Fang S. 2004 Which genetic marker for which conservation  
251 genetics issue? *Electrophoresis* **25**, 2165-2176. (doi:10.1002/elps.200305922)
- 252 26. Waterman JM. 1995 The social organization of the Cape ground squirrel (*Xerus inauris*;  
253 Rodentia: Sciuridae). *Ethology* **101**, 130-147. (doi:10.1111/j.1439-0310.1995.tb00352.x)
- 254 27. López-Darias M, Ribas A, Feliu C. 2008 Helminth parasites in native and invasive mammal  
255 populations: comparative study on the Barbary ground squirrel *Atlantoxerus getulus* L.  
256 (Rodentia, Sciuridae) in Morocco and the Canary Islands. *Acta Parasitol.* **53**, 296-301.  
257 (doi:10.2478/s11686-008-0036-5)
- 258 28. Piquet JC, López-Darias M, van der Marel A, Nogales M, Waterman J. 2018 Unraveling  
259 behavioral and pace-of-life syndromes in a reduced parasite and predation pressure context:  
260 personality and survival of the Barbary ground squirrel. *Behav. Ecol. Sociobiol.* **72**, 147.  
261 (doi:10.1007/s00265-018-2549-8)

- 262 29. van der Marel A, López-Darias M, Waterman JM. 2019 Group-enhanced predator detection  
263 and quality of vigilance in a social ground squirrel. *Anim. Behav.* **151**, 43–52.  
264 (doi:10.1016/j.anbehav.2019.02.017)
- 265 30. Jombart T. 2007 adegenet: a R package for the multivariate analysis of genetic markers.  
266 *Bioinformatics* **24**, 1403-1405. (doi:10.1093/bioinformatics/btn129)
- 267 31. Jombart T, Ahmed I. 2011 adegenet 1.3-1: new tools for the analysis of genome-wide SNP  
268 data. *Bioinformatics* **21**, 3070-3071. (doi:10.1093/bioinformatics/btr521)
- 269 32. Paradis E. 2010 pegas: an R package for population genetics with an integrated-modular  
270 approach. *Bioinformatics* **26**, 419-420. (doi:10.1093/bioinformatics/btp696)
- 271 33. R Core Team. 2018 R: A language and environment for statistical computing. R Foundation  
272 for Statistical Computing, Vienna, Austria. (URL: <https://www.R-project.org/>)
- 273 34. Dray S, Dufour A. 2007 The ade4 package: implementing the duality diagram for ecologists.  
274 *J. Stat. Softw.* **22**, 1-20. (doi:10.18637/jss.v022.i04)
- 275 35. Waples RS, Do C. 2008 LDNE: a program for estimating effective population size from data  
276 on linkage disequilibrium. *Mol. Ecol. Resour.* **8**, 753-756. (doi:10.1111/j.1755-  
277 0998.2007.02061.x)
- 278 36. van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004 Micro-Checker: software  
279 for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**,  
280 535-538. (doi:10.1111/j.1471-8286.2004.00684.x)
- 281 37. Dakin EE, Avise JC. 2004 Microsatellite null alleles in parentage analysis. *Heredity* **93**, 504-  
282 509. (doi:10.1038/sj.hdy.6800545)
- 283 38. Chybicki IJ, Burczyk J. 2009 Simultaneous estimation of null alleles and inbreeding  
284 coefficients. *J. Hered.* **100**, 106-113. (doi:10.1093/jhered/esn088)

- 285 39. Frankham R. 1995 Inbreeding and extinction: a threshold effect. *Conserv. Biol.* **9**, 792-799.  
286 (doi:10.1046/j.1523-1739.1995.09040792.x)
- 287 40. Crnokrak P, Roff DA. 1999 Inbreeding depression in the wild. *Heredity* **83**, 260-270.  
288 (doi:10.1038/sj.hdy.6885530)
- 289 41. Blackburn TM, Lockwood JL, Cassey P. 2015 The influence of numbers on invasion  
290 success. *Mol. Ecol.* **24**, 1942-1953. (doi:10.1111/mec.13075)
- 291 42. López-Darias M, Nogales M. 2008 Effects of the invasive Barbary ground squirrel  
292 (*Atlantoxerus getulus*) on seed dispersal systems of insular xeric environments. *J. Arid*  
293 *Environ.* **72**, 926-939. (doi:10.1016/j.jaridenv.2007.12.006)
- 294 43. Machado A, Domínguez F. 1982 *Estudio sobre la presencia de la Ardilla Moruna*  
295 (*Atlantoxerus getulus L.*) en la isla de Fuerteventura; su introducción, su biología y su  
296 impacto en el medio. Santa Cruz de Tenerife, Spain: Ministerio de Agricultura, Pesca  
297 y Alimentación. Instituto Nacional para la Conservación de la Naturaleza.
- 298 44. van der Marel A. 2019 Life history traits, social organisation and the drivers of sociality in an  
299 invasive ground squirrel. Doctoral dissertation, University of Manitoba, Winnipeg, MB.
- 300 45. Hedrick PW. 1994 Purging inbreeding depression and the probability of extinction: full-sib  
301 mating. *Heredity* **73**, 363-372. (doi:10.1038/hdy.1994.183)
- 302 46. Slade RW, Moritz C, Hoelzel AR., Burton HR. 1998 Molecular population genetics of the  
303 southern elephant seal *Mirounga leonina*. *Genetics* **149**, 1945-1957. (PMID:9691049)
- 304 47. Hückstädt L. 2015 *Mirounga angustirostris*. The IUCN Red List of Threatened Species.  
305 Version 2015. International Union for Conservation of Nature. Retrieved April 19, 2020.  
306 (doi:10.2305/IUCN.UK.2015-2.RLTS.T13581A45227116.en)

- 307 48. Kelly MJ. 2001 Lineage loss in Serengeti cheetah: consequences of high reproductive  
308 variance and heritability of fitness on effective population size. *Conserv. Biol.* **15**, 137-147.  
309 (doi:10.1111/j.1523-1739.2001.99033.x)
- 310 49. Durant S, Mitchell N, Ipavec A, Groom R. 2015 *Acinonyx jubatus*. The IUCN Red List of  
311 Threatened Species. Version 2015. International Union for Conservation of Nature.  
312 Retrieved July 16, 2019. (doi:10.2305/IUCN.UK.2015-4.RLTS.T219A50649567.en)
- 313 50. Tokarska M, Pertoldi C, Kowalczyk R, Perzanowski K. 2011 Genetic status of the European  
314 bison *Bison bonasus* after extinction in the wild and subsequent recovery. *Mammal Rev.* **41**,  
315 151-162. (doi:10.1111/j.1365-2907.2010.00178.x)
- 316 51. van der Marel A, Waterman JM, López-Darias M. 2020 Social organization in a North  
317 African ground squirrel. *J. Mamm.*, gyaa031. (doi:10.1093/jmammal/gyaa031)
- 318 52. Aulagnier S, Gouat P, Thévenot M. 2013 *Atlantoxerus getulus* *Barbary ground squirrel*, p.  
319 43-44. *In* Mammals of Africa: Volume III, ed D C D Happold. London: Bloomsbury  
320 Publishing.
- 321 53. Kumar S, Stecher G, Tamura K. 2015 MEGA7: Molecular Evolutionary Genetics Analysis  
322 version 7.0. *Mol. Biol. Evol.* **33**, 1870-1874. (doi:10.1093/molbev/msw054)
- 323 54. Detwiler JT, Zajac AM, Minchella DJ, Belden LK. 2012 Revealing cryptic parasite diversity  
324 in a definitive host: Echinostomes in muskrats. *J. Parasitol.* **98**, 1148-1155.  
325 (doi:10.1645/GE-3117.1)
- 326 55. Manjerovic MB, Wood PB, Edwards JW. 2009 Mast and weather influences on population  
327 trends of a species of concern: the Allegheny woodrat. *Am. Midl. Nat.* **162**, 52-61.  
328 (doi:10.1674/0003-0031-162.1.52)

- 329 56. Pompanon F, Bonin A, Bellemain E, Taberlet P. 2005 Genotyping errors: causes,  
330 consequences and solutions. *Nat. Rev. Genet.* **6**, 847-846. (doi:10.1038/nrg1707)
- 331 57. Hillis DM, Moritz C, Mable BK. 1996 *Nucleic Acids II: The Polymerase Chain Reaction*, p.  
332 238. *In* Molecular Systematics, 2nd ed. Sunderland: Sinauer Associates, Inc.
- 333 58. Stewart DT, Baker AJ. 1994 Patterns of sequence variation in the mitochondrial D-loop  
334 region of shrews. *Mol. Biol. Evol.* **11**, 9-21. (doi:10.1093/oxfordjournals.molbev.a040096)

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338 **Figures and Tables**

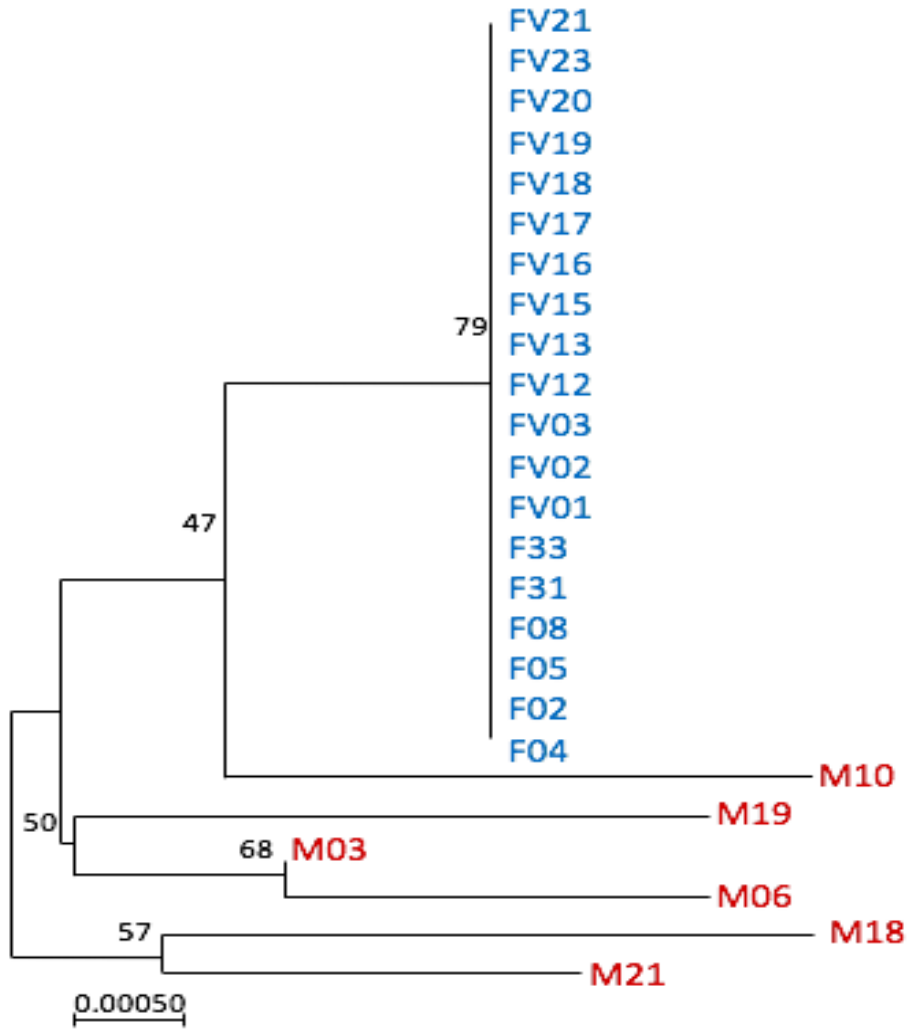
339 **Figure 1.** Evolutionary relationships between island (blue) and mainland (red) *Atlantoxerus*  
340 *getulus* based on the mitochondrial DNA displacement loop. Relationships inferred using the  
341 Neighbour-Joining method with 1000 bootstrap replicates. Evolutionary distances calculated  
342 using the Tamura-Nei method. All codon positions were included (total 1027 positions). Made in  
343 MEGA7 [53].  
344

345 **Figure 2.** Average inbreeding coefficients of 256 *Atlantoxerus getulus* individuals based on  
346 microsatellite markers of nuclear DNA. The  $F$  values ranged from 0.097 – 0.596 (mean  $F =$   
347 0.233).  
348

349 **Table 1.** Nucleotide differences within the mitochondrial d-loop of six *Atlantoxerus getulus*  
350 haplotypes from Sidi Ifni, Morocco.  
351

Position	27	95	98	111	114	173	252	258	276	282	289	299	316	746	824	970
M03	C	A	C	T	G	C	C	C	C	C	A	A	T	A	T	T
M06	.	.	.	.	.	.	T	.	.	.	.	.	.	G	.	.
M10	.	G	.	.	.	.	.	.	T	.	G	G	.	.	C	.
M18	T	.	.	C	.	.	.	.	.	.	.	.	C	.	C	C
M19	.	.	A	.	A	T	.	.	.	.	.	.	.	.	C	.
M21	.	G	.	.	.	.	.	T	.	.	.	.	C	.	C	.
FV	.	.	.	.	.	.	.	.	.	T	.	G	.	.	C	.

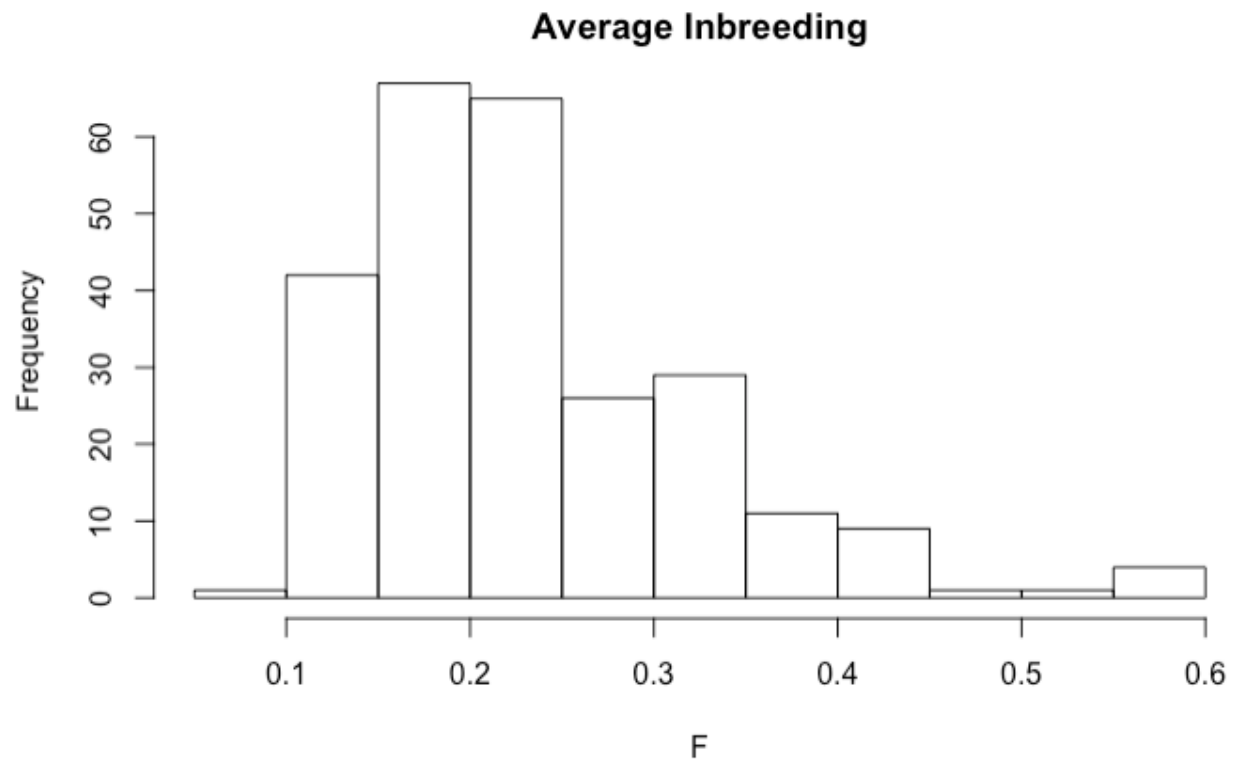
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Figure 1.

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Figure 2.

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