Title
Genomic Flatlining in the Endangered Island Fox

Journal
Current Biology, 26(9)

ISSN
09609822

Authors
Robinson, Jacqueline A
Ortega-Del Vecchyo, Diego
Fan, Zhenxin
et al.

Publication Date
2016-05-01

DOI
10.1016/j.cub.2016.02.062

Data Availability
The data associated with this publication are in the supplemental files.

Peer reviewed

Genomic flatlining in the endangered island fox

Jacqueline A. Robinson[1], Diego Ortega-Del Vecchyo[2], Zhenxin Fan[3], Bernard Y. Kim[1], Bridgett M. vonHoldt[4], Clare D. Marsden[1], Kirk E. Lohmueller[1, 2, 5]*, and Robert K. Wayne[1]*

Affiliations:

[1]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095, USA.

[2]Interdepartmental Program in Bioinformatics, University of California, Los Angeles, CA 90095, USA.

[3]Key Laboratory of Bioresources and Ecoenvironment (Ministry of Education), College of Life Sciences, Sichuan University, Chengdu 610064, People's Republic of China.

[4]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA.

[5]Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA.

Correspondence should be addressed to rwayne@ucla.edu

* These authors contributed equally to this work

Summary

Genetic studies of rare and endangered species often focus on defining and preserving genetically distinct populations, especially those having unique adaptations [1, 2]. Much less attention is directed at understanding the landscape of deleterious variation, an insidious consequence of geographic isolation and the inefficiency of natural selection to eliminate harmful variants in small populations [3-5]. With population sizes of many vertebrates decreasing, and isolation increasing through habitat fragmentation and loss, understanding the extent and nature of deleterious variation in small populations is essential for predicting and enhancing population persistence. The Channel Island fox (*Urocyon littoralis*) is a dwarfed species that inhabits six of California's Channel Islands, and is derived from the mainland gray fox (*U. cinereoargenteus*). These isolated island populations have persisted for thousands of years at extremely small population sizes [6, 7] and, consequently, are a model for testing ideas about the accumulation of deleterious variation in small populations under natural conditions. Analysis of complete genome sequence data from island foxes shows a dramatic decrease in genome-wide variation and a sharp increase in the homozygosity of deleterious variants. The San Nicolas Island population has a near absence of variation, demonstrating a unique genetic flatlining that is punctuated by heterozygosity hotspots, enriched for olfactory receptor genes and other genes with high levels of ancestral variation. These findings question the generality of the small population paradigm that maintains substantial genetic variation is necessary for short and long-term persistence.

Results and Discussion

To determine the extent of genetic variation in coding and non-coding regions in the island fox genome, and the role of demography and natural selection in shaping patterns of

variation, we sequenced genomes of seven island foxes representing each of the island populations and a mainland gray fox from southern California (Figures 1A, 1B). We included two genomes of the San Nicolas Island fox to better assess genome-wide patterns of variation because past research suggested a dramatic loss of variation in hypervariable loci [6, 8, 9]. Each of these island populations represents a morphologically and genetically distinct subspecies, four of which (San Miguel, Santa Rosa, Santa Cruz, Santa Catalina) have been listed as endangered under the US Endangered Species Act following catastrophic declines due to predation by non-native golden eagles (*Aquila chrysaetos*) and introduced canine distemper virus [10-12]. The individuals sequenced in this study were sampled in 1988, prior to the recent declines of these four populations. Each genome was sequenced with an Illumina HiSeq 2000 and aligned to the domestic dog reference genome, canFam3.1, followed by joint genotyping, filtering, and annotation of variants, yielding ~13-23X coverage (Table S1).

Genome-wide autosomal heterozygosity is high for the mainland gray fox (12.0 x 10^{-4}/bp), whereas heterozygosity in island foxes is reduced by 3- to 84-fold (4.08 - 0.142 x 10^{-4}/bp, Figures 1A, S1). The most extreme reduction of heterozygosity is found in the San Nicolas population (0.142 - 0.190 x 10^{-4}/bp), which has a genome that is almost entirely monomorphic (Figure 2A). Both San Nicolas individuals are nearly identical, differing at fewer than two sites per 100 kb. This remarkable absence of genomic variation in San Nicolas foxes is unprecedented in an outbreeding species (Figure 1C), though it is conceivable that our heterozygosity estimates could be biased downward by conservative data quality filters designed to mitigate the inclusion of sequencing errors. However, previous studies of hypervariable loci have consistently shown San Nicolas to be among the most monomorphic outbreeding animal populations [6, 8, 9]. After San Nicolas, the second most monomorphic fox genome is found on

San Miguel, the smallest of the fox-inhabited islands at 37 km^2. However, the San Miguel fox genome still has approximately seven times as many heterozygous sites as the San Nicolas foxes, although the populations have similar census sizes on the order of a few hundred individuals (Figure 1A) [6]. This finding suggests that, although a critical variable, long-term small population size does not completely account for the striking lack of diversity in San Nicolas foxes. Indeed, an extreme population bottleneck likely occurred in the San Nicolas population in the early 1970s [7, 13]. Nonetheless, the San Nicolas population subsequently rebounded without human assistance, despite the drastic reduction in genetic variation.

We used an approximate Bayesian computation (ABC) approach to estimate the parameters of demographic models that might account for the observed low heterozygosity in island populations. We utilized the distribution of heterozygous genotypes in a set of 13,647 1 kb putatively neutral regions distributed across the genome to infer demographic parameter values (see Supplemental Experimental Procedure). Based on previous genetic analyses [6-9, 14] and our inferred phylogenetic tree (Figure 1B), we assumed a bottleneck associated with the initial founding of the island fox, followed by island-specific colonization bottlenecks with no gene flow between islands or between the islands and the mainland (Figures S2, S3). We focused on modeling the demographic history of three populations: 1) the mainland gray fox, representing a population with no history of founder events or bottlenecks; 2) San Miguel, the smallest island population that nonetheless has greater diversity than the San Nicolas foxes, representing a population with small long-term effective size following island colonization; and 3) San Nicolas, the island population with the lowest observed heterozygosity, which has a history similar to that of San Miguel, but with a recent extreme bottleneck. These three models therefore exemplify the three major types of demographic history of individuals in our dataset (mainland, island,

93 island+bottleneck). We estimated a large effective population size equal to 5,185 during the last
94 500 generations in the gray fox, consistent with greater levels of variation in the large
95 outbreeding mainland population. In contrast, we found that the San Nicolas and San Miguel
96 populations had effective population sizes of 64 and 133 individuals, respectively, during more
97 than 93% of the last 500 generations (Figure S3). These results confirm that long-term small
98 population size is a key element explaining the extremely low variation observed in the island
99 populations, and that the exceptional lack of diversity in San Nicolas is the result of a recent
100 severe bottleneck.

101 Given that small population size and population bottlenecks, such as those inferred in the
102 island fox populations, are predicted to affect the efficacy of selection [3-5, 15], we assessed the
103 distribution of heterozygosity found in island fox genomes according to sequence context. We
104 used the annotation of the dog genome to extract sequences corresponding to putatively neutral
105 regions, exons of protein-coding genes, and conserved non-coding regions (Figure 3A). The
106 island genomes are characterized by decreased heterozygosity overall, however, with the
107 dramatic exception of San Nicolas, levels of variation match general expectations of reduced
108 heterozygosity in putatively functional (exonic and conserved non-coding) regions relative to
109 neutral regions. To more directly assess the efficacy of selection, we examined the ratio of
110 heterozygosity of zero- to four-fold degenerate sites (zero-fold degenerate sites: all mutations are
111 non-synonymous, four-fold degenerate sites: all mutations are synonymous). This ratio is
112 predicted to be elevated in small populations, since deleterious alleles can increase in frequency
113 under strong drift and weakened selection [15, 16]. Consistent with this prediction, we found a
114 negative relationship between the ratio of zero-fold to four-fold heterozygosity and neutral
115 heterozygosity, with smaller populations having lower neutral heterozygosity but higher zero-

116 fold heterozygosity (Figure 3B). San Nicolas is the most extreme in this regard with highly
117 elevated levels of putatively deleterious zero-fold heterozygosity. Using forward in time
118 population genetic simulations, we found that our demographic models, combined with a
119 distribution of fitness effects inferred from human polymorphism data [17], are sufficient to
120 explain the observed ratios (Figure 3B). Therefore demographic history can account for the
121 observed increase of deleterious heterozygosity in the island genomes, demonstrating the long-
122 term effects of small population size on reducing the efficacy of selection in small, isolated
123 populations.
124 To further investigate the consequences of weaker selection in the island populations, we
125 annotated variants within coding regions with respect to their effect on the amino acid sequence,
126 and polarized alleles as ancestral or derived using the dog as an outgroup (Figure 3C). We
127 utilized the prediction algorithm *Sorting Intolerant From Tolerant* (*SIFT*) [18], which estimates
128 if missense mutations are likely to be damaging by assessing evolutionary constraint in
129 homologous protein alignments, with the assumption that mutations observed at positions highly
130 conserved across taxa are likely to disrupt function (DEL: *SIFT* score<0.05), whereas others are
131 more likely to be tolerated (TOL: *SIFT* score≥0.05). We also catalogued loss-of-function
132 mutations, as these mutations have a high probability of impacting fitness by reducing or
133 eliminating gene functionality [19, 20]. In the mainland gray fox, genotypes containing
134 putatively derived alleles (heterozygous and homozygous derived) are lowest for deleterious
135 variants (loss-of-function and deleterious) relative to benign variants (tolerated and
136 synonymous), demonstrating that purifying selection in the large mainland population effectively
137 removes deleterious variation (Figure 3C). In contrast, all island populations have reduced
138 heterozygosity and an elevation of homozygosity for derived alleles, suggesting the conversion

of deleterious alleles from the heterozygous to the homozygous state through strong genetic drift reducing the efficacy of purifying selection. The number of missense derived deleterious alleles per individual is 6.2% higher in the island populations than in the gray fox (P<0.007, Table S2) suggesting a greater additive genetic load in the island populations. Additionally, the island foxes have more than twice the number of homozygous loss-of-function genotypes compared to the mainland fox (P<1.75 x 10^{-5}, Figure 3C, Table S2), implying a substantial genetic load, particularly if loss-of-function variants are recessive [21, 22]. The general increase in loss-of-function alleles in all the island populations indicates that the accumulation and fixation of deleterious variants is a feature associated with long-term small population size and isolation, rather than a recent extreme bottleneck as observed in the San Nicolas population. In combination, these results argue that small population sizes have not resulted in more efficient purging of deleterious alleles, and have instead significantly increased the genetic load of the island populations.

A previous genotyping study of five major histocompatibility complex (MHC) loci in the San Nicolas population found evidence for balancing selection [7]. However, coverage limitations and the frequent presence of pseudogenes in our sequence data complicate assessments of heterozygosity at the MHC region. Consequently, we instead searched for regions of elevated heterozygosity throughout the genome at sites passing our series of quality filters. Within each genome, peaks greater than two standard deviations above the mean heterozygosity in a sliding window analysis across the autosomes were considered to be elevated (Figures 2A, S1). We found 66 and 48 discrete peaks in the San Nicolas genomes (526 - 1,092 peaks in other island fox genomes, 763 peaks in the gray fox genome). We found that peak regions were not enriched for genic content (P≥0.595), but were significantly enriched for a variety of gene

ontology (GO) terms (Table S3). In the two San Nicolas fox sequences and the mainland gray fox, we found enrichment of olfactory receptor (OR) genes (*KEGG:04740*) in heterozygosity peaks (San Nicolas 1: $P=3.20 \times 10^{-20}$, San Nicolas 2: $P=5.68 \times 10^{-4}$, gray fox: $P=1.16 \times 10^{-15}$). A result this extreme rarely occurs by chance (San Nicolas 1: $P=10^{-3}$, San Nicolas 2: $P=0.033$, gray fox: $P<10^{-3}$). However, OR genes commonly occur in clusters, a spatial factor that is not formally considered in the GO enrichment test that could inflate apparent enrichment. Additionally, OR genes have high ancestral levels of variation, as suggested by their presence in peaks in the gray fox and high rates of OR gene polymorphism observed in mammals [23-25].

To assess whether these peaks of heterozygosity remaining in the San Nicolas genome could be the result of neutral demographic processes, we conducted simulations of 1,000 full genomes under our San Nicolas demographic model. We find that the simulated genomes contain as many or more peaks of similar magnitude, suggesting that peaks of heterozygosity are expected even after severe bottlenecks, demonstrating that balancing selection is not necessary to account for the pattern of heterozygosity observed in the San Nicolas genomes (Figures 2B, 2C). Therefore, the remaining heterozygosity peaks in the San Nicolas genomes appear to be the remnants of high heterozygosity in the ancestral population that has yet to be eliminated through drift, rather than being actively maintained through balancing selection. This effect is more likely to occur in regions of the genome that have elevated polymorphism in the ancestral population, such as olfactory receptor genes. As we have shown in the San Nicolas fox, consideration of demographic models is essential to determine if the number of high heterozygosity peaks could be due to demographic history and drift alone.

We show that island fox populations have experienced a dramatic reapportionment of deleterious variation and have two to three times more variants in the homozygous state for loss-

185 of-function and deleterious missense variants. In the San Nicolas Island fox, heterozygosity has
186 flatlined genome-wide such that the population has a near absence of genetic variation, with
187 remaining variation evident in only a few genes with high initial levels of variation, such as the
188 OR gene family. This lack of variation can be explained by an extremely small effective size of
189 about 64 individuals over 500 generations ago, followed by a severe bottleneck ~30 generations
190 ago that reduced the population to fewer than a dozen individuals. The dominant effect of
191 demography across all island populations is a reduction in the efficacy of selection and a
192 consequent increase in the load of deleterious variation. Nonetheless, the island populations
193 appear healthy, can recover from disease epidemics, and the four recently bottlenecked
194 populations recovered rapidly under human management after introduced non-native disease or
195 predation threats were removed [26, 27]. The unaided persistence of the San Nicolas population,
196 and the successful recovery of the four endangered populations, contrasts with other cases in
197 which loss of genetic diversity following population declines has resulted in apparent inbreeding
198 depression, hampering recovery [28, 29]. The long-term persistence of island foxes despite their
199 small population sizes and increased genetic load presents a challenge for the small population
200 conservation paradigm [30], which emphasizes population size and its effects on genetic
201 variation as crucial factors in long-term persistence or endangerment. The absence of obvious
202 negative effects on population persistence from genetic deterioration may in part reflect a more
203 benign island environment, given the lack of competitors and predators that exist on the
204 mainland. If island environments are more benign in general, then island populations may often
205 tolerate higher levels of genetic load than mainland counterparts. Notably, our results contradict
206 the notion that long-term small effective population size and inbreeding on the islands have
207 enhanced purging and decreased their genetic load [31]. Conceivably, phenotypic plasticity

mediated by regulatory and epigenetic mechanisms may help compensate for the lack of genomic variation, a possibility that can now be explored in small populations using new and developing molecular techniques [32, 33].

Experimental Procedures

Samples and sequencing

DNA samples representing each of the Channel Island fox populations and one mainland gray fox from southern California were used for whole genome sequencing on an Illumina HiSeq 2000. Island fox DNA samples were originally obtained for a population genetic study in 1988, prior to subsequent population declines due to predation and disease in four of the island populations [8]. Samples for sequencing were selected to maximize DNA quality and quantity, evaluated by gel electrophoresis, NanoDrop spectrophotometer, and Qubit fluorometer. Each individual was sequenced with at least one lane of paired-end 100 bp reads. Sample and sequence information is summarized in Table S1.

Alignment and annotation

Reads were trimmed and filtered for quality before being aligned to the domestic dog genome (canFam3.1). Over 90% of reads successfully aligned to the dog reference genome yielding ~13-23X coverage per individual (Table S1). We applied conservative ad hoc filters to minimize the inclusion of erroneous genotypes. We used the genomic coordinates identified in Freedman et al. [34] to extract coding, conserved non-coding, and putatively neutral sequences. Briefly, coordinates of 196,668 exons were obtained from a filtered set of transcripts originating from NCBI, Ensembl, and UCSC. 319,958 mammalian conserved non-coding sequences were identified from a multi-genome alignment of 11 Euarchontoglires as regions longer than 50 bp

with a phastCons score > 0.7 [35]. The putatively neutral regions comprise a set of 13,647 1 kb loci located at least 30 kb apart, at least 10 kb from coding sequence, and 100 bp from conserved non-coding sequence while avoiding regions of the genome with repeats, poor assembly, or low mappability. Coordinates of zero-fold and four-fold degenerate sites within coding transcripts were those identified as positions where mutations would always or never change the encoded amino acid, respectively [36]. Variant annotation was performed with *VEP* [37] running *SIFT* [18], using Ensembl's *C. familiaris* annotation database (release 78), to identify loss-of-function, deleterious (*SIFT* score<0.05), tolerated (*SIFT* score≥0.05), and synonymous mutations. Loss-of-function mutations were defined as those that encoded a premature stop codon. Further details are provided in the Supplemental Experimental Procedure.

Heterozygosity peak analysis

Heterozygosity was calculated as the fraction of heterozygous genotypes of all genotypes passing filters in 100 kb windows with a 10 kb step size. Heterozygosity "peaks" were identified as windows with heterozygosity in excess of two standard deviations above the mean, calculated per genome (Fig. S1). Coordinates of peaks of heterozygosity are given in Data S2. Heterozygosity peak regions were tested for enrichment of genic content by tallying the number of bases within them that overlapped with genes, and testing whether this proportion was significantly higher than expected by chance. Gene ontology (GO) enrichment analysis was performed on peak coordinates using *gProfileR* with the Ensembl *C. familiaris* annotation (release 79) [38].

Demographic inference and simulations

We used approximate Bayesian computation (ABC) to infer the demographic history of the San Nicolas, San Miguel, and mainland gray fox populations (Figures S3B, S3C).

Demographic models and prior distributions of parameters were based on estimates from our own analyses (Figures 1B, S2, S3A) and the literature [7, 14]. We conducted forward in time simulations with a modified version of the forward simulator used in Lohmueller 2014 [39] to jointly assess the effects of demography and purifying selection on levels of deleterious variation. We conducted simulations with *MaCS* [40] to determine whether the observed number of high heterozygosity peaks observed in the San Nicolas genome is expected under neutrality using our inferred demographic model. Full descriptions of these methods are provided in the Supplemental Experimental Procedure.

Accession Numbers

Sequence data are available at NCBI's Sequence Read Archive under BioProject PRJNA312115.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, three tables, and two datasets and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.02.062.

Author Contributions

JAR and RKW conceived and designed the experiment. Sequence analysis was primarily carried out by JAR with assistance from DODV, ZF, BYK, BMvH, CDM, and KEL. Demographic inference and simulations were performed by DODV. The manuscript was written by JAR, DODV, KEL, and RKW, with input from all authors.

Acknowledgements

We acknowledge support from a QCB Collaboratory Postdoctoral Fellowship to CDM, and the QCB Collaboratory community directed by Matteo Pellegrini. KEL is supported by a Searle Scholars Fellowship and an Alfred P. Sloan Research Fellowship in Computational & Molecular Biology. This work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 Instrumentation Grants S10RR029668 and S10RR027303 and a UC President's Catalyst award.

285 **References**

286 1. Crandall, K.A., Bininda-Emonds, O.R., Mace, G.M. and Wayne, R.K. (2000). Considering
287 evolutionary processes in conservation biology. Trends Ecol. Evol. *15*, 290-295.
288 2. Funk, W.C., McKay, J.K., Hohenlohe, P.A. and Allendorf, F.W. (2012). Harnessing genomics
289 for delineating conservation units. Trends Ecol. Evol. *27*, 489-496.
290 3. Lande, R. (1994). Risk of population extinction from fixation of new deleterious mutations.
291 Evolution *48*, 1460-1469.
292 4. Lynch, M., Conery, J. and Burger, R. (1995). Mutation Accumulation and the Extinction of
293 Small Populations. Am. Nat. *146*, 489-518.
294 5. Kirkpatrick, M. and Jarne, P. (2000). The effects of a bottleneck on inbreeding depression and
295 the genetic load. Am. Nat. *155*, 154–167.
296 6. Wayne, R.K., George, S.B., Gilbert, D., Collins, P.W., Kovach, S.D., Girman, D. and
297 Lehman, N. (1991). A morphologic and genetic study of the island fox, *Urocyon*
298 *littoralis*. Evolution *45*, 1849-1868.
299 7. Aguilar, A., Roemer, G., Debenham, S., Binns, M., Garcelon, D. and Wayne, R.K. (2004).
300 High MHC diversity maintained by balancing selection in an otherwise genetically
301 monomorphic mammal. Proc. Natl Acad. Sci. *101*, 3490-3494.
302 8. Gilbert, D.A., Lehman, N., O'Brien, S.J. and Wayne, R.K. (1990). Genetic fingerprinting
303 reflects population differentiation in the California Channel Island fox. Nature *344*, 764-
304 767.
305 9. Goldstein, D.B., Roemer, G.W., Smith, D.A., Reich, D.E., Bergman, A. and Wayne, R.K.
306 (1999). The use of microsatellite variation to infer population structure and demographic
307 history in a natural model system. Genetics *151*, 797-801.

10. Roemer, G.W., Coonan, T.J., Garcelon, D.K., Bascompte, J. and Laughrin, L. (2001). Feral pigs facilitate hyperpredation by golden eagles and indirectly cause the decline of the island fox. Anim. Conserv. *4*, 307-318.

11. Timm, S.F., Munson, L., Summers, B.A., Terio, K.A., Dubovi, E.J., Rupprecht, C.E., Kapil, S. and Garcelon, D.K. (2009). A suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes (*Urocyon littoralis catalinae*). J. Wildl. Dis. *45*, 333-343.

12. United States Fish and Wildlife Service (2004). Listing of the San Miguel Island fox, Santa Rosa Island fox, Santa Cruz Island fox, and Santa Catalina Island fox as endangered; final rule. Federal Register *69*, 10335–10353.

13. Laughrin, L. L. (1980). Population and status of the island fox. In The California Islands: Proceedings of a Multidisciplinary Symposium, D.M. Powers, ed. (Santa Barbara, CA: Santa Barbara Museum of Natural History), pp. 745–750.

14. Hofman, C.A., Rick, T.C., Hawkins, M.T., Funk, W.C., Ralls, K., Boser, C.L., Collins, P.W., Coonan, T., King, J.L., Morrison, S.A. et al. (2015). Mitochondrial genomes suggest rapid evolution of dwarf California Channel Islands foxes (*Urocyon littoralis*). PloS one *10*, e0118240.

15. Sousa, V., Peischl, S. and Excoffier, L. (2014). Impact of range expansions on current human genomic diversity. Curr. Opin. Genet. Devel. *29*, 22-30.

16. Lohmueller, K.E., Indap, A.R., Schmidt, S., Boyko, A.R., Hernandez, R.D., Hubisz, M.J., Sninsky, J.J., White, T.J., Sunyaev, S.R., Nielsen, R. et al. (2008). Proportionally more deleterious genetic variation in European than in African populations. Nature *451*, 994-997.

17. Boyko, A.R., Williamson, S.H., Indap, A.R., Degenhardt, J.D., Hernandez, R.D., Lohmueller, K.E., Adams, M.D., Schmidt, S., Sninsky, J.J., Sunyaev, S.R. et al. (2008). Assessing the evolutionary impact of amino acid mutations in the human genome. PLoS Genet. *4*, e1000083.

18. Kumar, P., Henikoff, S. and Ng, P.C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat. Protoc. *4*, 1073-1081.

19. MacArthur, D.G., Balasubramanian, S., Frankish, A., Huang, N., Morris, J., Walter, K., Jostins, L., Habegger, L., Pickrell, J.K., Montgomery, S.B. et al. (2012). A systematic survey of loss-of-function variants in human protein-coding genes. Science *335*, 823-828.

20. Sulem, P., Helgason, H., Oddson, A., Stefansson, H., Gudjonsson, S.A., Zink, F., Hjartarson, E., Sigurdsson, G.T., Jonasdottir, A., Jonasdottir, A. et al. (2015). Identification of a large set of rare complete human knockouts. Nat. Genet. *47*, 448-452.

21. Kimura, M., Maruyama, T. and Crow, J.F. (1963). The mutation load in small populations. Genetics *48*, 1303-1312.

22. Peischl, S. and Excoffier, L. (2015). Expansion load: recessive mutations and the role of standing genetic variation. Mol. Ecol. *24*, 2084-2094.

23. Alonso, S., Lopez, S., Izagirre, N. and de la Rua, C. (2008). Overdominance in the human genome and olfactory receptor activity. Mol. Biol. Evol. *25*, 997-1001.

24. Chen, R., Irwin, D.M. and Zhang, Y.P. (2012). Differences in selection drive olfactory receptor genes in different directions in dogs and wolf. Mol. Biol. Evol. *29*, 3475-3484.

25. Gilad, Y., Bustamante, C.D., Lancet, D. and Pääbo, S. (2003). Natural selection on the

olfactory receptor gene family in humans and chimpanzees. Am. J. Hum. Genet. *73*, 489-501.

26. Coonan, T.J., Bakker, V., Hudgens, B., Boser, C.L., Garcelon, D.K. and Morrison, S.A. (2014). On the fast track to recovery: island foxes on the northern Channel Islands. Monogr. West. N. Am. Nat. *7*, 373-381.

27. King, J.L., Duncan, C.L. and Garcelon, D.K. (2014). Status of the Santa Catalina Island fox thirteen years after its decline. Monogr. West. N. Am. Nat. *7*, 382-396.

28. Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L. and Paige, K.N (1998). Tracking the long-term decline and recovery of an isolated population. Science *282*, 1695-1698.

29. Johnson, W.E., Onorato, D.P., Roelke, M.E., Land, E.D., Cunningham, M., Belden, R.C., McBride, R., Jansen, D., Lotz, M., Shindle, D. et al. (2010). Genetic restoration of the Florida panther. Science *329*, 1641-1645.

30. Caughley, G. (1994). Directions in conservation biology. J. Anim. Ecol. *63*, 215-244.

31. Xue, Y., Prado-Martinez, J., Sudmant, P.H., Narasimhan, V., Ayub, Q., Szpak, M., Frandsen, P., Chen, Y., Yngvadottir, B., Cooper, D.N. et al. (2015). Mountain gorilla genomes reveal the impact of long-term population decline and inbreeding. Science *348*, 242-245.

32. Kristensen, T.N., Pedersen, K.S., Vermeulen, C.J. and Loeschcke, V. (2010). Research on inbreeding in the 'omic' era. Trends Ecol. Evol. *25*, 44-52.

33. Vergeer, P., Wagemaker, C.A.M. and Ouborg, N.J. (2012). Evidence for an epigenetic role in inbreeding depression. Biol. Lett. *8*, 798-801.

34. Freedman, A.H., Gronau, I., Schweizer, R.M., Ortega-Del Vecchyo, D., Han, E., Silva, P.M., Galaverni, M., Fan, Z., Marx, P., Lorente-Galdos, B. et al. (2014). Genome sequencing

highlights the dynamic early history of dogs. PLoS Genet. *10*, e1004016.

35. Siepel, A., Bejerano, G., Pedersen, J.S., Hinrichs, A.S., Hou, M., Rosenbloom, K., Clawson, H., Spieth, J., Hillier, L.W., Richards, S. and Weinstock, G.M. (2005). Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Res. *15*, 1034-1050.

36. Marsden, C.D., Ortega-Del Vecchyo, D., O'Brien, D.P., Taylor, J.F., Ramirez, O., Vilà, C., Marques-Bonet, T., Schnabel, R.D., Wayne, R.K. and Lohmueller, K.E. (2016). Bottlenecks and selective sweeps during domestication have increased deleterious genetic variation in dogs. Proc. Natl Acad. Sci. *113*, 152-157.

37. McLaren, W., Pritchard, B., Rios, D., Chen, Y., Flicek, P. and Cunningham, F. (2010). Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics *26*, 2069-2070.

38. Reimand, J., Kull, M., Peterson, H., Hansen, J. and Vilo, J. (2007). g: Profiler—a web-based toolset for functional profiling of gene lists from large-scale experiments. Nucleic Acids Res. *35*, W193-W200.

39. Lohmueller, K.E. (2014). The impact of population demography and selection on the genetic architecture of complex traits. PLoS Genet. *10*, e1004379.

40. Chen, G.K., Marjoram, P. and Wall, J.D. (2009). Fast and flexible simulation of DNA sequence data. Genome Res. *19*, 136-142.

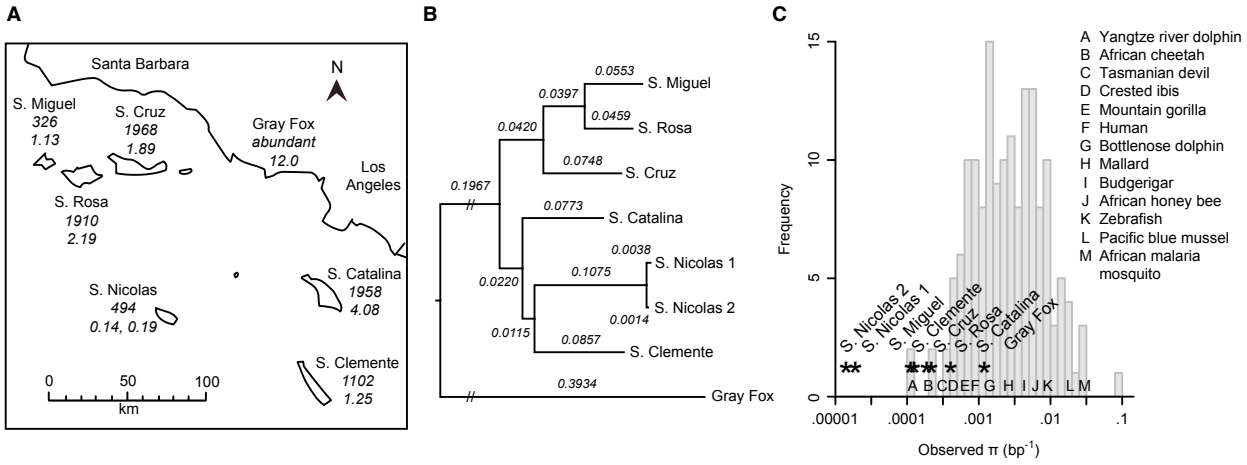

Figure 1. Island foxes show exceptionally low heterozygosity. (A) Map showing island geography, estimated census size at time of sampling (upper number) [6], and genome-wide heterozygosity per 10 kb of autosomal sequence (lower number). See also Figure S1. (B) Neighbor-joining tree constructed from a genome-wide pairwise distance matrix, displaying reciprocal monophyly of northern and southern island populations, and the southern California gray fox as the outgroup. Genetic distance is indicated on the branches. All nodes have 100% bootstrap support. See also Figure S2. (C) Histogram showing the distribution of published genome-wide estimates of π from 159 outbreeding species (137 animal, 11 plant, 8 fungus, and 3 protist taxa; see Supplemental Experimental Procedure), with the position of island and gray fox heterozygosity values indicated by asterisks. See also Data S1.

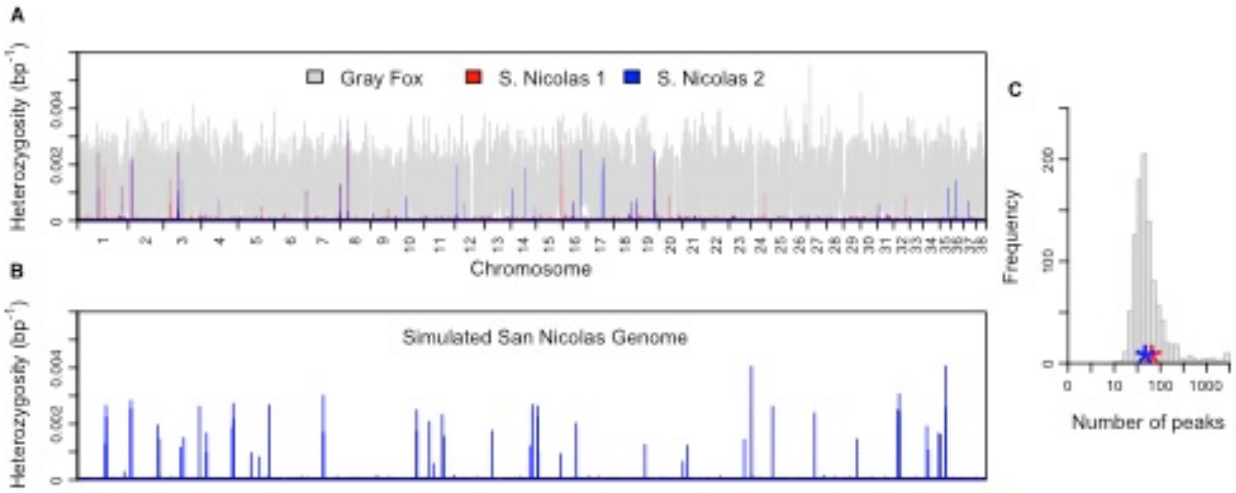

Figure 2. Distribution of peaks of heterozygosity across the island and mainland fox genomes. (A) Empirical heterozygosity per 100 kb window with a 10 kb step size across the genome in two San Nicolas foxes (red and blue lines, joint purple lines) and the mainland gray fox (gray lines). The mainland fox exhibits heterozygosity across the entire genome, whereas the San Nicolas foxes have virtually no heterozygosity except at a few distinct peaks. See also Figure S1. (B) An example of a simulated genome using the San Nicolas population demographic model, showing a similar lack of heterozygosity genome-wide except at a few distinct peaks. According to these simulations, peaks of equivalent magnitude and number can be generated by genetic drift alone. See also Figure S3. (C) Histogram showing that the observed number of peaks in the San Nicolas genomes (colored asterisks) falls near the mode of the distribution of the number of peaks generated in genomes simulated under the San Nicolas demographic model (P = 0.257-0.451). See also Data S2.

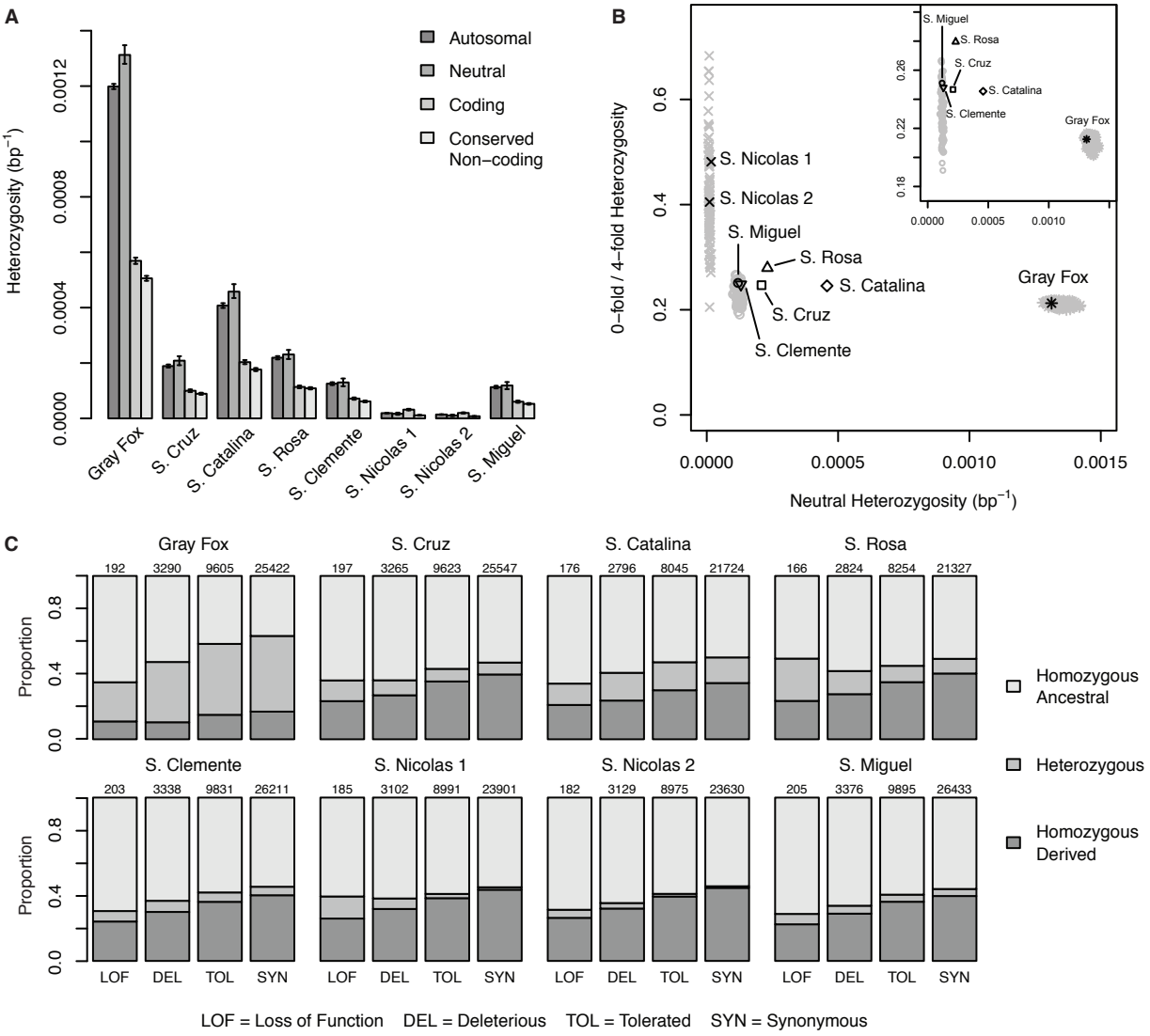

Figure 3. Extreme drift in island foxes reapportions deleterious genetic variation. (A) In all foxes, except San Nicolas individuals, autosomal and neutral diversity is higher than in regions putatively under purifying selection (coding and conserved non-coding sequence). Error bars represent 95% confidence intervals determined from bootstrap resampling. Individuals are ordered according to census sizes (Figure 1A). (B) The negative relationship between neutral diversity (influenced by effective population size), and the ratio of heterozygosity at zero-fold relative to four-fold sites (influenced by the efficacy of purifying selection). Light gray symbols represent values obtained from simulations using the distribution of selective effects inferred in

humans [17] and our demographic models (Figure S3). A negative relationship between the zero-fold/four-fold heterozygosity ratio and neutral diversity also persists after excluding the San Nicolas foxes (inset). At extremely low effective population size, as in San Nicolas, there is high variance in the ratio of zero-fold/four-fold heterozygosity in simulations due to low counts of heterozygous genotypes. (C) Proportion of genotypes in each individual that are homozygous ancestral, heterozygous, or homozygous derived (with respect to the dog reference genome) at segregating sites within coding regions. Total genotype counts are indicated at the tops of bars for each category. Loss-of-function mutations are those that encode a premature stop codon, whereas "deleterious" and "tolerated" missense mutations are categorized by *SIFT* [18]. See also Table S2.