

Supplemental Information

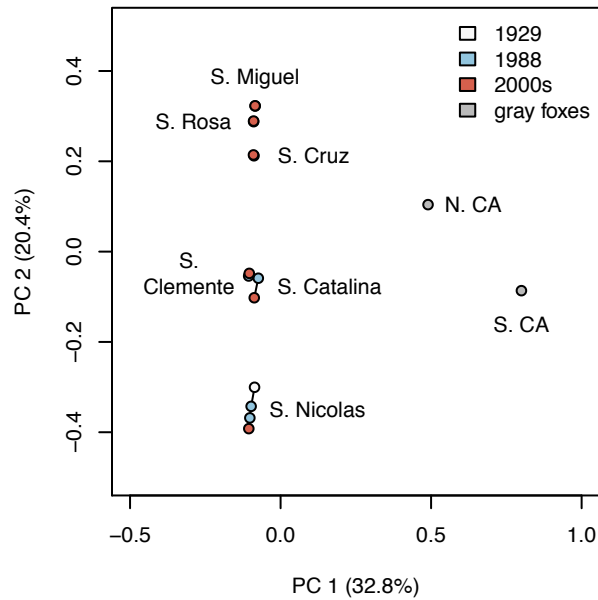


Figure S1. Principal component analysis of gray and island fox genomes. Related to Figure 1. Based on 12,249 SNPs pruned for linkage disequilibrium. Solid black lines connect points from the same population.

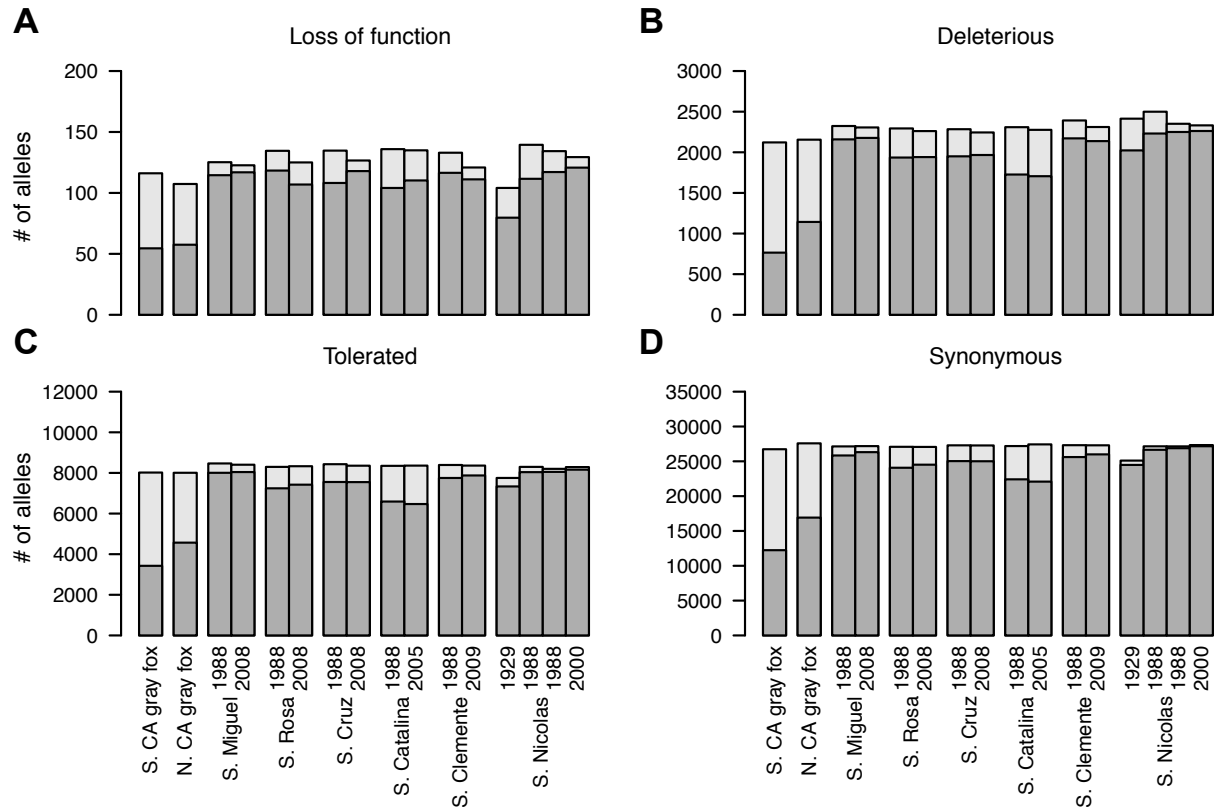


Figure S2. Number of derived alleles in island and gray fox coding regions. Related to Figure 2. The number of derived alleles contained in homozygous genotypes in each individual is shown in dark grey, and the total number of derived alleles in light grey. The homozygosity and total number of derived alleles is elevated at putatively deleterious variant sites in island fox genomes (see Table S4). Mutations are classified by annotation type by the Ensembl Variant Effect Predictor [VEP; S1] and the Sorting Intolerant From Tolerant algorithm [SIFT; S2]. Numbers within each category are normalized by the mean number of genotyped sites across individuals to account for differences in coverage between individuals. (A) Loss of function mutations are those that encode premature stop codons. (B, C) Deleterious and tolerated mutations are missense mutations categorized by SIFT [S2] according to whether they are predicted to be damaging. (D) Synonymous changes are mutations that do not change the encoded amino acid and are presumed to be neutral.

Species	Location	Sample #	Sex	Year Sampled	Platform	Base Pairs (Gbp)	# of reads aligned, post-filtering ($\times 10^6$)	Mean depth of coverage (X)	Source
ULI	Santa Catalina	SCA16/RKW4644	F	1988	HiSeq2000, 2x100bp	82.5	311	12.8	[S3]
ULI	San Clemente	SCLV4/RKW4045	F	1988	HiSeq2000, 2x100bp	73.2	450	18.7	[S3]
ULI	Santa Cruz	SCZ05/RKW12331	M	1988	HiSeq2000, 2x100bp	77.9	348	14.5	[S3]
ULI	San Miguel	SMI15/RKW12354	F	1988	HiSeq2000, 2x100bp	80.5	559	23.2	[S3]
ULI	San Nicolas	SNI05/RKW4038	F	1988	HiSeq2000, 2x100bp	66.1	339	13.8	[S3]
ULI	San Nicolas	SNI41/RKW12349	F	1988	HiSeq2000, 2x100bp	84.3	328	13.6	[S3]
UCI	SMMNRA	NPS GFO41	F	2012	HiSeq2000, 2x100bp	67.5	409	17.0	[S3]
ULI	Santa Catalina	RKW11697	M	2005	HiSeq4000, 2x100bp	70.8	526	22.3	This study
ULI	San Clemente	RKW13704	F	2009	HiSeq4000, 2x100bp	69.5	442	18.7	This study
ULI	Santa Cruz	RKW8695	F	2008	HiSeq4000, 2x100bp	72.3	511	21.7	This study
ULI	San Miguel	RKW11655	M	2008	HiSeq4000, 2x100bp	69.6	516	21.9	This study
ULI	San Nicolas	RKW12297	M	2000	HiSeq4000, 2x100bp	73.0	492	20.9	This study
ULI	San Nicolas	Dickey 15477	F	1929	HiSeq4000, 1x100bp	118.5	478	15.8	This study
ULI	Santa Rosa	SRO13/RKW12355	F	1988	HiSeq4000, 2x100bp	76.0	527	22.2	This study
ULI	Santa Rosa	RKW10660	M	2008	HiSeq4000, 2x100bp	78.0	534	22.7	This study
UCI	GOGANRA	NPS GFO30	M	1993	HiSeq4000, 2x100bp	70.6	446	18.8	This study

Table S1. Sample information and metrics for genome sequences. Related to Figure 1. ULI: *Urocyon littoralis*; UCI: *Urocyon cinereoargenteus*; SMMNRA: Santa Monica Mountains National Recreation Area; GOGANRA: Golden Gate National Recreation Area. In the main text and supplemental items, San Nicolas 1988 (1) refers to SNI05/RKW4038 and San Nicolas 1988 (2) refers to SNI41/RKW12349. All raw sequence reads are available for download from the NCBI Sequence Read Archive. Sequences from Robinson et al. 2016 [S3] can be found under BioProject PRJNA312115, and sequences generated for this study can be found under PRJNA478450.

Statistic	Empirical value	Empirical percentile	Simulated mean	Simulated 2.5th percentile	Simulated 97.5th percentile
Number of peaks in 1929 genome	122	89.2	99.7	22	498
Number of peaks in 1988 (1) genome	140	91.2	98.9	20	655
Number of peaks in 1988 (2) genome	94	87.7	95.4	20	729
Number of peaks in 2000 genome	92	86.9	97	20	644
Mean peak width in 1929 genome	204.5 kb	13.1	312.7 kb	134.5 kb	570.5 kb
Mean peak width in 1988 (1) genome	186.0 kb	20.8	289.2 kb	127.9 kb	549.7 kb
Mean peak width in 1988 (2) genome	216.4 kb	19.3	294.6 kb	128.8 kb	607.1 kb
Mean peak width in 2000 genome	199.5 kb	20.2	289.3 kb	128.6 kb	539.2 kb
Proportion of shared peaks in 1929 genome	0.415	15.9	0.537	0.293	0.761
Proportion of shared peaks in 1988 (1) genome	0.687	27.3	0.731	0.311	0.919
Proportion of shared peaks in 1988 (2) genome	0.869	86.7	0.731	0.344	0.939
Proportion of shared peaks in 2000 genome	0.754	46.9	0.732	0.312	0.918
Proportion of peaks shared by 2+ individuals among all 4 San Nicolas genomes	0.439	15.4	0.729	0.346	0.789

Table S2. Statistics related to peaks of heterozygosity in empirical and simulated San Nicolas fox genomes. Related to Figure 1. No empirical values fall outside of the middle 95% of values obtained through simulation.

Individual	Enrichment p-value	GO Term	Description
S. CA Gray Fox (1932 genes in 861 peaks)	1.29E-16	GO:0050911	Detection of chemical stimulus involved in sensory perception of smell
	1.46E-11	GO:0007186	G-protein coupled receptor signaling pathway
	6.84E-11	GO:0005886	Plasma membrane
	0.00576	GO:0045095	Keratin filament
	0.0163	GO:0000786	Nucleosome
	0.00556	GO:0005549	Odorant binding
	1.29E-16	GO:0004984	Olfactory receptor activity
	0.0103	HP:0005356	Decreased serum complement factor I
	0.0432	KEGG:04610	Complement and coagulation cascades
N. CA Gray Fox (2897 genes in 1109 peaks)	1.43E-16	KEGG:04740	Olfactory transduction
	0.014	GO:0018101	Protein citrullination
	0.00108	GO:0071944	Cell periphery
	3.90E-13	GO:0005882	Intermediate filament
	0.00317	GO:0016021	Integral component of membrane
	0.014	GO:0004668	Protein-arginine deiminase activity
	0.0209	GO:0060089	Molecular transducer activity
S. Catalina 1988 (3539 genes in 1213 peaks)	0.00498	KEGG:04740	Olfactory transduction
	0.0477	GO:0098662	Inorganic cation transmembrane transport
	0.0331	GO:0045095	Keratin filament
S. Catalina 2005 (3830 genes in 1349 peaks)	0.000906	GO:0016021	Integral component of membrane
	1.23E-29	GO:0050906	Detection of stimulus involved in sensory perception
	0.00278	GO:1900543	Negative regulation of purine nucleotide metabolic process
	1.65E-05	GO:0005882	Intermediate filament
	1.32E-13	GO:0005886	Plasma membrane
	4.66E-06	GO:0005549	Odorant binding
	1.52E-29	GO:0004984	Olfactory receptor activity
	0.00587	GO:0004064	Arylesterase activity
	3.64E-25	KEGG:04740	Olfactory transduction
S. Clemente 1988 (2697 genes in 643 peaks)	0.00371	KEGG:04610	Complement and coagulation cascades
	NA	NA	NA
S. Clemente 2009 (2342 genes in 617 peaks)	NA	NA	NA
S. Cruz 1988 (3630 genes in 1203 peaks)	0.00413	KEGG:04924	Renin secretion
	0.0295	KEGG:04713	Circadian entrainment
S. Cruz 2008 (3575 genes in 1253 peaks)	0.0222	GO:0007399	Nervous system development
	0.027	GO:0048856	Anatomical structure development
	0.0143	GO:0008146	Sulfotransferase activity
S. Miguel 1988 (2506 genes in 614 peaks)	NA	NA	NA

S. Miguel 2008 (1966 genes in 431 peaks)	5.56E-06	GO:0045095	Keratin filament
	0.0454	HP:0010669	Hypoplasia of the zygomatic bone
	0.0428	HP:0002253	Colonic diverticula
	0.0106	HP:0004428	Elfin facies
	0.0129	HP:0000796	Urethral obstruction
	0.00157	HP:0100025	Overfriendliness
	0.001	HP:0002623	Overriding aorta
	0.042	HP:0010780	Hyperacusis
	0.00157	HP:0001361	Nystagmus-induced head nodding
	0.042	HP:0100817	Renovascular hypertension
	0.000385	HP:0007720	Flat cornea
	0.0227	HP:0000015	Bladder diverticulum
	0.0221	HP:0002183	Phonophobia
S. Nicolas 1929 (531 genes in 122 peaks)	2.55E-07	GO:0007186	G-protein coupled receptor signaling pathway
	5.38E-17	GO:0007608	Sensory perception of smell
	5.59E-17	GO:0004984	Olfactory receptor activity
	0.00578	GO:0005549	Odorant binding
	3.57E-16	KEGG:04740	Olfactory transduction
S. Nicolas 1988 (1) (626 genes in 140 peaks)	0.0229	GO:0035095	Behavioral response to nicotine
	5.61E-31	GO:0007186	G-protein coupled receptor signaling pathway
	1.15E-43	GO:0050907	Detection of chemical stimulus involved in sensory perception
	3.79E-12	GO:0016021	Integral component of membrane
	0.000464	GO:0004252	Serine-type endopeptidase activity
	2.36E-05	GO:0003823	Antigen binding
	0.00943	GO:0004497	Monooxygenase activity
	0.0126	GO:0016705	Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen
	2.94E-43	GO:0004984	Olfactory receptor activity
	0.0259	GO:0005549	Odorant binding
8.42E-31	KEGG:04740	Olfactory transduction	
S. Nicolas 1988 (2) (440 genes in 94 peaks)	8.05E-19	GO:0007608	Sensory perception of smell
	0.0036	GO:0035095	Behavioral response to nicotine
	0.00871	GO:0016021	Integral component of membrane
	0.021	GO:0005886	Plasma membrane
	1.16E-18	GO:0004984	Olfactory receptor activity
	2.21E-09	GO:0005149	Interleukin-1 receptor binding
	0.0241	GO:0004252	Serine-type endopeptidase activity
	0.0496	HP:0002654	Multiple epiphyseal dysplasia
	2.78E-15	KEGG:04740	Olfactory transduction
S. Nicolas 2000 (379 genes in 92 peaks)	0.00689	GO:0051716	Cellular response to stimulus
	7.45E-26	GO:0050907	Detection of chemical stimulus involved in sensory perception
	6.62E-19	GO:0007186	G-protein coupled receptor signaling pathway

	0.0253	GO:0031224	Intrinsic component of membrane
	7.46E-09	GO:0005549	Odorant binding
	0.000212	GO:0004252	Serine-type endopeptidase activity
	7.43E-25	GO:0004984	Olfactory receptor activity
	3.79E-20	KEGG:04740	Olfactory transduction
S. Rosa 1988 (3715 genes in 1235 peaks)	0.00932	KEGG:00590	Arachidonic acid metabolism
S. Rosa 2008 (3735 genes in 1209 peaks)	0.018	GO:0044763	Single-organism cellular process
	0.0218	GO:0051216	Cartilage development
	0.0389	GO:0048149	Behavioral response to ethanol
	0.000344	GO:0005887	Integral component of plasma membrane
	0.0329	GO:0004499	N,N-dimethylaniline monooxygenase activity
	0.000802	KEGG:04080	Neuroactive ligand-receptor interaction

Table S3. Gene ontology enrichment results for peaks of heterozygosity in island and gray fox genomes. Related to Figure 1. Only terms with significant enrichment p -values (<0.05) are shown.

Type	Null model MLE	Alternative model MLE	Likelihood ratio test
Proportion of Homozygous Derived Genotypes (Gray Foxes v. 1988-2009 Island Foxes)			
Synonymous	$q_i=q_g=0.320$	$q_i=0.339, q_g=0.196$	$\Lambda=6692.54, p<<10^{-16}$
Tolerated	$q_i=q_g=0.279$	$q_i=0.298, q_g=0.157$	$\Lambda=2480.20, p<<10^{-16}$
Deleterious	$q_i=q_g=0.208$	$q_i=0.224, q_g=0.104$	$\Lambda=808.49, p<<10^{-16}$
Loss of function	$q_i=q_g=0.234$	$q_i=0.251, q_g=0.124$	$\Lambda=40.44, p=2.03 \times 10^{-10}$
Proportion of Derived Alleles (Gray Foxes v. 1988-2009 Island Foxes)			
Tolerated	$q_i=1.002435q_g, q_g=0.326$	$q_i=0.328, q_g=0.315$	$\Lambda=31.60, p=1.89 \times 10^{-8}$
Deleterious	$q_i=1.002435q_g, q_g=0.251$	$q_i=0.254, q_g=0.234$	$\Lambda=32.72, p=1.06 \times 10^{-8}$
Loss of function	$q_i=1.002435q_g, q_g=0.282$	$q_i=0.289, q_g=0.247$	$\Lambda=6.76, p=9.33 \times 10^{-3}$
Proportion of Derived Alleles (Gray Foxes v. 1988 Island Foxes)			
Tolerated	$q_i=1.001165q_g, q_g=0.325$	$q_i=0.328, q_g=0.315$	$\Lambda=30.20, p=3.90 \times 10^{-8}$
Deleterious	$q_i=1.001165q_g, q_g=0.252$	$q_i=0.254, q_g=0.234$	$\Lambda=40.56, p=1.91 \times 10^{-10}$
Loss of function	$q_i=1.001165q_g, q_g=0.285$	$q_i=0.289, q_g=0.247$	$\Lambda=8.53, p=3.50 \times 10^{-3}$
Proportion of Derived Alleles (Gray Foxes v. 2000-2009 Island Foxes)			
Tolerated	$q_i=1.003915q_g, q_g=0.324$	$q_i=0.328, q_g=0.315$	$\Lambda=25.40, p=4.66 \times 10^{-7}$
Deleterious	$q_i=1.003915q_g, q_g=0.246$	$q_i=0.254, q_g=0.234$	$\Lambda=18.24, p=1.95 \times 10^{-5}$
Loss of function	$q_i=1.003915q_g, q_g=0.271$	$q_i=0.289, q_g=0.247$	$\Lambda=3.67, p=5.56 \times 10^{-2}$

Table S4. Comparisons of the proportion of homozygous derived genotypes and the proportion of derived alleles per individual between gray and island foxes. Related to Figure 2. Significant p -values (<0.05) are in bold.

	S. Catalina	S. Clemente	S. Cruz	S. Miguel	S. Nicolas	S. Rosa	Gray Fox
S. Catalina		2.00	<i>Inf</i>	2.58	7.33	2.50	3.75
S. Clemente	1.60		NA	1.55	3.20	1.30	1.88
S. Cruz	<i>Inf</i>	<i>Inf</i>		0	0	0	0
S. Miguel	10.33*	7.75*	0		2.06	0.640	1.03
S. Nicolas	1.30	0.977	0	0.176*		0.312	2.13
S. Rosa	NA	NA	NA	<i>Inf</i>	<i>Inf</i>		0.660
Gray Fox	<i>Inf</i> *	<i>Inf</i> *	NA	0	0	NA	

Table S5. Relative risks of developmental and probable collision pathologies between island foxes and mainland gray fox morphological samples. Related to Figure 3. Relative risks of developmental pathologies are above the diagonal and relative risks of probable collision pathologies are below the diagonal. The table is read: *Row* has X times the risk of pathology as *Column*. A zero represents no risk relative to the column; "*Inf*" represents cases where the column had no reported cases and the risk in the row population is thus infinitely greater. For collision pathologies, a comparison is not available (NA) between samples where neither sample contained probable collision pathologies. Asterisks denote risks significant below a 0.05 threshold based on the permutation test. For developmental pathologies, no relative risk results were statistically significant.

Selection Strength (s)	N_{island}	Additive Regime					Recessive Regime					Fold-change	
		Split	Big Island	Ancient Bottleneck	Serial Bottleneck	Recent Bottleneck	Inbreeding	Split	Big Island	Ancient Bottleneck	Serial Bottleneck		Recent Bottleneck
[-1, -0.1)	1000	0.875	1.14	1.15	1.10	0.963	0.984	0.400	0.395	0.391	0.314	0.255	0.138
	500	1.06	1.27	0.941	1.07	0.904	0.648	0.259	0.268	0.217	0.251	0.201	0.0836
	200	1.19	1.30	0.850	1.05	0.718	1.61	0.131	0.176	0.154	0.182	0.0965	0.0777
	100	0.646	0.812	1.79	0.971	0.630	1.14	0.143	0.0882	0.0677	0.0549	0.145	0.0492
	50	0.450	1.97	1.60	1.91	3.15	2.47	0.0799	0.127	0.122	0.0750	0.0507	0.0172
[-0.1, -0.01)	1000	1.03	0.953	1.00	1.05	1.17	1.03	0.330	0.349	0.335	0.330	0.341	0.266
	500	1.17	1.02	1.08	1.03	1.29	0.928	0.241	0.238	0.267	0.260	0.253	0.216
	200	2.19	1.69	2.04	2.24	2.67	1.95	0.316	0.255	0.304	0.286	0.298	0.233
	100	9.55	8.39	9.05	9.16	11.0	9.89	1.04	0.742	0.905	0.905	0.906	0.962
	50	26.6	22.8	27.6	29.8	28.2	29.1	2.36	1.95	2.39	2.25	2.26	2.45
[-0.01, -0.001)	1000	1.74	1.63	1.87	1.88	1.76	1.74	0.630	0.605	0.651	0.682	0.609	0.605
	500	3.51	3.18	3.29	3.58	3.31	3.25	1.02	0.867	1.01	0.993	0.998	1.00
	200	7.03	6.16	6.82	7.00	6.72	6.57	1.74	1.42	1.75	1.79	1.77	1.81
	100	9.65	8.36	9.46	9.48	10.3	9.73	2.34	1.82	2.30	2.36	2.27	2.26
	50	11.3	9.87	11.8	12.1	11.8	12.1	2.66	2.20	2.71	2.67	2.61	2.63
[-0.001, 0)	1000	1.09	1.07	1.13	1.11	1.07	1.10	1.05	1.03	1.05	1.04	1.03	1.02
	500	1.11	1.12	1.12	1.12	1.12	1.10	1.07	1.06	1.08	1.08	1.08	1.06
	200	1.13	1.12	1.14	1.14	1.13	1.16	1.11	1.09	1.10	1.09	1.11	1.11
	100	1.13	1.12	1.12	1.13	1.13	1.13	1.10	1.09	1.10	1.11	1.09	1.11
	50	1.15	1.12	1.15	1.15	1.15	1.15	1.10	1.09	1.11	1.11	1.12	1.11
[-1, 0)	1000	1.10	1.08	1.14	1.12	1.08	1.11	1.00	0.989	1.01	0.997	0.987	0.976
	500	1.15	1.14	1.15	1.16	1.14	1.13	1.05	1.03	1.06	1.05	1.05	1.03
	200	1.22	1.19	1.22	1.22	1.21	1.23	1.13	1.09	1.12	1.12	1.14	1.14
	100	1.25	1.23	1.24	1.25	1.26	1.25	1.18	1.13	1.18	1.19	1.16	1.18
	50	1.32	1.27	1.33	1.34	1.33	1.33	1.23	1.18	1.24	1.24	1.24	1.24
0	1000	1.01	1.01	0.988	1.01	1.01	1.01	1.00	1.00	1.00	0.995	1.00	1.01
	500	1.01	1.01	0.997	1.02	1.01	0.997	1.01	1.00	1.01	1.02	0.999	1.00
	200	1.01	0.995	0.997	0.993	0.983	1.01	1.01	1.01	0.992	0.987	0.990	0.993
	100	1.02	0.995	1.01	1.00	0.994	1.00	1.01	0.993	0.985	0.996	0.983	0.987
	50	0.987	0.996	1.01	0.997	1.00	0.979	0.999	0.996	1.01	1.00	1.01	1.00

Table S6. Results from forward simulations of neutral and deleterious variation with different island population sizes. Related to Figure 4. Pictorial depictions of demographic models are shown in Figure 4A of the main text. The value in each cell represents the mean fold-change in the total number of alleles per individual in the island population relative to the mainland. For example, a value of 1.00 indicates no difference in the average number of alleles between the island and mainland, a value of 0.50 indicates that island individuals have half as many alleles relative to the mainland on average, and a value of 2.00 indicates that island individuals have twice as many alleles relative to the mainland on average. Cells are color-coded according to their value, such that blue indicates a depletion (fold-change <1.00) and red indicates an enrichment (fold-change >1.00) of the number of alleles per individual on the island relative to the mainland. The enrichment color intensity is capped at 2-fold enrichment. Values that are significant ($p < 0.05$) are bold and italicized.

Supplemental References

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- S2. 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.
- S3. Robinson, J.A., Ortega-Del Vecchyo, D., Fan, Z., Kim, B.Y., Marsden, C.D., Lohmueller, K.E. and Wayne, R.K. (2016). Genomic flatlining in the endangered island fox. *Curr. Biol.* 26, 1183-1189.