Supplementary Materials for:

Genetic monitoring of the greater stick-nest rat meta-population for strategic translocation planning

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Supplementary Methods: DNA Extraction

Tissue samples stored in ethanol were air dried for 45 minutes prior to digestion. Samples were digested overnight at 55°C in 300 µL of lysis buffer (10mM Tris, 0.1M EDTA pH8 and 2% SDS), 60 µg of proteinase K, and 0.08 M dithiothreitol (DTT). Digested samples were incubated at 37°C with 1 µL of RNase A (10 mg/ml; Thermo Scientific) for 30 minutes. After digestion, 100 µL of 7.5 M ammonium acetate was added, the mixture was vortexed and left on ice for one hour. The samples were then centrifuged at 13,000 rpm for 5 minutes and the pellet was discarded. The supernatant was mixed with 300 µl of 100% isopropanol and 0.5 µl of glycogen (20mg/ml, Sigma-Aldrich) and placed on ice for approximately one hour. The mixture was mixed gently by inversion and then spun at 15,000 rpm for 10 minutes. The supernatant was discarded and the pellet washed in 300 µL of 70% ethanol and then air dried for 30 minutes. The DNA pellet was re-suspended at 65°C for one hour in 40 µL of TLE buffer (10 mM Tris, 0.1 M EDTA, pH 8). DNA extracts were quantified using the Quantus Fluorometer system (Promega) as per the manufacturer's instructions.

Supplementary Methods: ddRAD-seq library Preparation

Digestion and ligation reactions were performed in 96-well plates, each including a library blank. We digested 300 ng of each DNA extract at 37 °C for 2 hours using 8 U of the restriction endonucleases *PstI* and *HpaII* in 20 µL of 1× CutSmart Buffer and H_2O (New England Biosciences [NEB]). *PstI* is a rare cutting enzyme with a six-base recognition site (CTGCAG) and *HpaII* is a more common cutting enzyme with a four-base recognition site (CCGG).

We then ligated uniquely barcoded adapters (see below and SM Table 2) to the sticky ends of the digested fragments. Ligation reactions were performed in 40 µL volumes consisting of 20 µl of digested DNA, 200 U of T4 ligase, 0.1 ρmol of forward (rare) and 15

ρmol of reverse (common) adapters, 1× T4 Buffer and H_2O. The mixture was left at room temperature for 2 hours, and then heat killed at 65°C for 20 minutes. We pooled the ligation products into 12 libraries of eight samples each. Pooled libraries were purified using the QIAquick PCR purification kit (Qiagen) and eluted in 120 µL of EB buffer (Qiagen).

PCR reactions to add the full-length Illumina adapters were performed in eight replicates per library in 30 µL volumes containing 10 µL of purified library, 1× Hot Start Taq Master Mix (NEB), 0.66 µM each of the forward and reverse primers (SI Figure 1) and H_2O. The PCR conditions were as follows: 95° C for 30 seconds, 16 cycles of 95° C for 30 seconds, 65° C for 20 seconds, and 68° C for 30 seconds, followed by 68° C for 5 minutes, and 25° C for 1 minute. The eight replicates per library were re-pooled and purified as above, eluting in 30 µL of EB buffer (Qiagen). We employed a two-step double-SPRI protocol to select for fragments between 100 and 300 bp using SPRI beads.

Supplementary Methods: Design and preparation of barcoded adapters

Both the barcoded forward primer and the common reverse primer (a Y-adapter) were designed as per Poland *et al.* (2012). A set of 96 barcodes were designed using the barcode-generator python script (https://github.com/audy/barcode-generator) to range in size from 5–9 bp in length, with a Levenstein distance of at least 3 to allow samples to be distinguished from one another even with one sequencing error in each barcode (see SI methods). The single-stranded oligonucleotides of each barcode adapter and the common adapter were resuspended to 100 µM in 1× Elution Buffer (EB; 10mM Tris-Cl, pH=8.0). To make a plate of working aliquots for the double stranded adapters, we added 10 µl of each single stranded oligo (at 100 µM) to 10 µl of 10× Adapter Buffer (AB; 500mM NaCl, 100mM Tris-Cl) and 70 µl of H_2O. This mixture was then heated to 95°C for 2 minutes, cooled at 1°C per minute until 30° C was reached, and then held at 4°C for 5 minutes. The barcoded adapters were then

diluted 3:10 with AB and quantified using Quant-iT Picogreen dsDNA dye (Invitrogen) on a Quantus fluorometer (Promega Corporation). Each barcoded adapter was normalised to 1.6 ng/µl (=0.1µM). A plate containing a combination of the forward barcoded adapter and common reverse adapter was then prepared by adding 20 µl of the barcoded adapter (at 0.1 µM) to 30 µl of the common reverse adapter (at 10 µM) and 50 µl of 1× AB.

Supplementary Methods: STACKS work flow and parameters

Firstly, raw sequencing reads were de-multiplexed, truncated to 65 bp, and filtered for overall quality based on the presence of barcodes using the *process_radtags* module. Next, RAD loci were identified for each sample using the *ustacks* module, requiring a minimum stack read depth of three (m = 3) and a maximum of two nucleotide mismatches (M = 2) between sequences within a stack. Loci with more than three stacks (mls = 3) and more reads than two standard deviations above the mean were filtered as they may map to multiple points on the genome or represent non-biallelic sites. A 'deleveraging algorithm' was used to try to resolve over-merged loci. A catalogue of consensus loci among individuals was constructed with the *cstacks* module using the *ustacks* output files. Loci were recognized as homologous across individuals if there were two or fewer mismatches between the consensus sequences (*n* = 2). Alleles were identified in each individual against this catalogue using the module *sstacks*.

References

Davis-Richardson A. (2015) GitHub respository. https://github.com/audy/barcode-generator.

Poland, J.A., Brown, P.J., Sorrells, M.E. & Jannink, J.-L. (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE* **7**, e32253.

SM Table 1. Glossary of the main measures which were estimated from our data. Definitions are taken from Frankham, Ballou and Briscoe (2010). Figures and/or tables which present these measures in the main text are given in parentheses.

Measure	Abbreviation	Definition/Interpretation
Allelic Richness	A_R	A measure of genetic diversity. Higher values indicate higher diversity. The average number of alleles per locus per population. We use a calculation of this measure that corrects for sample size. (Table 2)
Observed Heterozygosity	H_O	A measure of genetic diversity. Higher values indicate higher diversity. The proportion of heterozygous individuals for a locus in a population. We present this measure as an average per population. (Table 2)
Expected Heterozygosity	H_E	A measure of genetic diversity. Higher values indicate higher diversity. The heterozygosity expected for a random mating population with the given allele frequencies according to the Hardy-Weinberg equilibrium. We present this measure as an average per population. (Table 2)
Individual Inbreeding Coefficient	F	A measure of the extent of inbreeding in an individual (i.e. a measure of the relatedness of an individual's parents). Higher values indicate a greater extent of inbreeding. The probability that two alleles at a locus in an individual are identical by descent. (Figure 2)
Genetic Relatedness	genetic R	A measure of genetic similarity between two individuals. Higher values indicate greater genetic similarity. Can also be thought of as the individual inbreeding coefficient of two individuals' hypothetical offspring. We present this measure calculated in two different ways. In the first instance we calculate genetic R using the method of Hedrick and Lacy (2015), estimating the proportion of the genome that is identical by descent between two individuals. We use this method to identify and remove close relatives within each population (SM Figure 1). In the second instance we use the method of Wang (2002) to estimate genetic similarity between all pairs of individuals in our entire sample, presenting the estimates as within and between population averages (Figure 3 and Table 3, respectively).
Pairwise F_{ST}	F_{ST}	A measure of genetic divergence between two (sub)populations. Higher values indicate greater genetic divergence. The proportion of the total inbreeding in a population due to differentiation between two sub-populations. (Table 3)
Effective Population Size	Ne	The population size which ultimately leads to varying genetic outcomes. Smaller values will lead to more rapidly accumulating adverse genetic outcomes. The number of individuals that would result in the same loss of genetic diversity, inbreeding or genetic drift if they behaved in the manner of an idealized population. Real populations deviate in structure from an idealized population in many ways, and therefore Ne is almost always smaller than the census size. (Table 4)

SM Table 2: Break-down of details of the individuals transferred from the remnant population on the Franklin Island to the Monarto captive colony. Sex ratios are given as male to female (M:F). These details were compiled from an unpublished Adelaide Zoo report.

Year	**Number of individuals transferred from West Franklin Island (M:F)**	**Number of individuals added from East Franklin Island (M:F)**	**Total Added (M:F)**
1985	3:1	0:0	3:1
1986	0:1	0:0	0:1
1988	4:4	3:1	7:5
1990	0:0	0:1	0:1
1991	0:0	3:0	3:0
1994	0:0	3:5	3:5
Total	7:6	9:7	16:13

SM Table 3. Permit numbers for all live animal trapping conducted as part of this study.

Location	Permit Number	Permit From
Arid Recovery	58-2015	South Australian Wildlife Ethics Committee
St Peter Island	U26475-1	South Australian Wildlife Ethics Committee
Salutation Island	2016-34	Western Australian Department of Parks and Wildlife Animal Ethics Committee
Mt Gibson	2014-20	Western Australian Department of Parks and Wildlife Animal Ethics Committee

SM Table 4. Unique barcodes used for each double-digest RAD library, shown here in 96-well plate format.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CTCGAGT	GTGTGAGT	GAGTATG	GACTCT	AGACGAGA	CCGTCGCCA	GCAGA	CGAGAG	TCCGCCGCA	GCTCG	ACGATG	GTACGT
B	AGCACTA	TGCATGCGT	CATGGCCGA	CTATCG	GAACAAGT	ACACT	TGATCTA	AGGCGACCT	ACCTACCG	GTATTCTAT	CAGCAGA	GCCAT
C	GTCGATG	TCACGAGT	AATGGACA	ACTCGCCA	TCAGATG	TGGCAA	ATGTACT	CCGCGTCCA	ATCACTAT	ATCAGAG	CTCAT	TACTGATAG
D	GTCGTACTG	ATGGCG	CGTATGCT	CGAGCGT	TGCCACCA	TACTACAG	CCGGACTGA	GAGCGTCGT	TACGA	ATACACAG	CTGAAGAA	TTCCGA
E	TCTCTCA	CGTGATGCA	CACTGAG	TGCAGCG	AGTGACAA	ATCTGTCT	GTCTG	CAGTATAGA	TAGATGTA	GCATCAGCG	CATCCG	ATTCGG
F	TATGATTCT	TAGCGTG	AGCAGTCT	TCTTATAGT	GTACGCTGT	CTATA	TCACTG	GGCTAG	ATGTCA	GTGTGCAT	ACTACGTG	CGTCGA
G	CCGATGGCA	CACTCG	TGTATAT	TTGTTAACT	ACCTG	TATCT	AGTATAGT	TGTAG	TCATGG	TTAATCGTT	CGCGCATA	CTGCG
H	AGCTA	GTGCGATAG	ACGTGTACT	ACTCTA	GCTGACG	ACGAGTG	ATCTGTA	TGCTAT	TCGTACTA	CAGATA	TAGGCT	AGCCGTGCA

SM Table 5. Sequencing results of samples used in this study. * denote samples removed from dataset due to the presence of close relatives.

Sample	Population	Reads	Number of Loci	Number of SNPs	Percent Missing Data	Average Depth of Coverage
321	Arid Recovery	1747771	91034	6219	28.71	11.99
282	Arid Recovery	2624274	116537	7229	17.13	16.74
284	Arid Recovery	2722355	116790	7996	8.33	17.12
2197	Arid Recovery	3258362	149041	7673	12.04	15.48
294	Arid Recovery	5189201	170074	8500	2.56	24.50
347	Arid Recovery	3576200	136011	7957	8.78	19.74
559*	Arid Recovery	3724920	156128	8314	4.69	17.88
430*	Arid Recovery	4361914	154409	8307	4.77	22.20
435	Arid Recovery	2663562	112107	7906	9.37	16.39
646	Arid Recovery	4750963	174597	8527	2.25	21.01
560*	Arid Recovery	3471059	144980	8191	6.10	17.73
493	Arid Recovery	6622181	211613	8600	1.41	24.48
516*	Arid Recovery	8386909	234469	8640	0.95	29.24
597*	Arid Recovery	6077728	204073	8617	1.22	23.62
328	Arid Recovery	3699057	148902	8262	5.28	18.50
513	Arid Recovery	8031913	201222	8624	1.13	32.80
530	Arid Recovery	4380176	168614	8362	4.14	19.86
LC1439	East Franklin	2424943	110503	6763	22.47	14.86
LC1438	East Franklin	1998033	93870	6327	27.47	15.07
LC1441	East Franklin	6280569	221856	8591	1.51	21.72
LC1440	East Franklin	3657452	149893	8091	7.25	17.49
LC1445	East Franklin	3182399	146615	7952	8.84	15.42
LC1444	East Franklin	4734105	185412	8384	3.89	18.87
LC1421	East Franklin	5457258	195919	8577	1.67	20.98
LC1447	East Franklin	4312959	170101	8327	4.54	19.05
6047	Monarto	1796858	90768	6332	27.41	13.57
6048	Monarto	2142067	102406	6974	20.05	14.54
6046	Monarto	2361385	109678	7179	17.70	15.49
6045	Monarto	2710666	121883	7856	9.94	16.00
6049	Monarto	2258007	93039	7485	14.19	17.36
6044	Monarto	4005091	144880	8207	5.92	21.21
77E8C54*	Mt Gibson	1902887	93857	6448	26.08	12.98
7A0149F*	Mt Gibson	13377179	274184	8643	0.92	41.15
7A3C855	Mt Gibson	3037446	145336	7674	12.03	14.68
7A2E993	Mt Gibson	5054695	195981	8341	4.38	19.73
74D8EA2	Mt Gibson	4317118	169354	8507	2.48	18.86
74D970C	Mt Gibson	9826289	253282	8659	0.73	31.57
79F7AA0	Mt Gibson	3193030	137322	8069	7.50	16.43
77E8974	Mt Gibson	8200164	221372	8618	1.20	30.20
609A	Reevesby Island	1586931	81627	5749	34.09	12.57
6011	Reevesby Island	1810268	80475	6889	21.02	15.24

6306	Reevesby Island	962517	45656	3977	54.41	13.14
6305	Reevesby Island	1341994	71600	5156	40.89	11.86
6324	Reevesby Island	928256	41942	3616	58.55	13.79
6019	Reevesby Island	1109242	57961	5050	42.11	10.78
6313*	Reevesby Island	1552939	71955	5473	37.26	13.79
6017	Reevesby Island	1374737	70029	5645	35.29	12.52
6331	Reevesby Island	1323380	69248	6024	30.94	12.15
6006	Reevesby Island	2121320	106741	7199	17.47	13.81
6332	Reevesby Island	2229417	105942	7007	19.67	14.73
6327	Reevesby Island	2752424	118017	7485	14.19	16.71
6328	Reevesby Island	1668713	85246	6030	30.87	13.07
6031	Reevesby Island	2657781	121889	7660	12.19	15.92
6003	Reevesby Island	2720249	115613	7511	13.89	17.31
6314	Reevesby Island	2629099	130001	7570	13.22	14.28
6319	Reevesby Island	3504089	128088	7253	16.85	20.72
6042*	Reevesby Island	5846226	186009	8573	1.72	24.93
6020	Reevesby Island	2146368	101834	7551	13.44	13.94
6009	Reevesby Island	3933136	146984	8159	6.47	20.70
6308	Reevesby Island	3418055	119844	7208	17.37	21.97
6041	Reevesby Island	3502000	140178	8184	6.18	18.81
6337	Reevesby Island	1492285	65974	4204	51.81	15.40
6325	Reevesby Island	2430544	112683	7263	16.74	15.26
6010	Reevesby Island	7235658	194627	8520	2.33	29.59
6338	Reevesby Island	2264804	91728	6749	22.63	17.94
6033	Reevesby Island	4395750	151217	8340	4.39	21.95
6304	Reevesby Island	2439437	98427	7491	14.12	18.19
6035	Reevesby Island	4338173	161604	8457	3.05	20.17
6039*	Reevesby Island	2776634	116126	7292	16.40	16.98
6016*	Reevesby Island	4429305	162149	8524	2.28	20.65
6309	Reevesby Island	3664626	140247	7283	16.51	19.65
6326	Reevesby Island	3506182	129863	7819	10.36	19.93
6301	Reevesby Island	5957349	175793	8541	2.09	26.90
6018*	Reevesby Island	2512222	109692	7913	9.29	15.73
6334	Reevesby Island	8585082	229603	8644	0.91	29.68
6322	Reevesby Island	3147220	127573	7637	12.45	18.25
6333*	Reevesby Island	5485231	176274	8360	4.16	24.95
6037	Reevesby Island	3306814	143647	8142	6.66	16.80
6001	Reevesby Island	3562713	138711	7858	9.92	19.02
6329	Reevesby Island	5241695	164683	8392	3.79	25.72
6318	Reevesby Island	3749760	140996	8264	5.26	19.90
6302	Reevesby Island	7946053	201326	8581	1.63	31.85
6023	Reevesby Island	4735540	173311	8530	2.21	21.18
6310	Reevesby Island	4343418	141167	7712	11.59	24.12
6330	Reevesby Island	4670781	134939	8133	6.76	27.20
6317	Reevesby Island	5069803	179199	8580	1.64	22.32

6021	Reevesby Island	3342776	143707	8081	7.36	17.25
6002*	Reevesby Island	4822016	168566	8522	2.30	21.76
6339	Reevesby Island	5186259	166083	8341	4.38	24.62
6014	Reevesby Island	6516891	191091	8485	2.73	27.35
6022	Reevesby Island	5063856	170874	8368	4.07	22.66
6038	Reevesby Island	2980655	123523	8049	7.73	17.31
6312	Reevesby Island	6712318	217950	8616	1.23	24.44
6040	Reevesby Island	4844842	169300	8492	2.65	22.51
6036	Reevesby Island	2727863	116844	8049	7.73	16.46
6027	Reevesby Island	6672776	193934	8617	1.22	27.24
6004	Reevesby Island	6874160	196716	8603	1.38	27.51
6015	Reevesby Island	5279965	162513	8331	4.49	25.43
6335	Reevesby Island	4463745	160044	8396	3.75	21.05
6043	Reevesby Island	7014082	194629	8605	1.35	28.91
6341	Reevesby Island	3221365	122277	8147	6.60	19.19
6311	Reevesby Island	5743541	185296	8418	3.50	24.09
6007	Reevesby Island	11527763	279400	8650	0.84	33.60
6026	Reevesby Island	5361895	179446	8585	1.58	23.68
6303	Reevesby Island	5164334	165274	8472	2.88	24.60
6024	Reevesby Island	3830691	135381	8237	5.57	20.86
6030	Reevesby Island	5978084	174431	8551	1.97	27.29
6340	Reevesby Island	9380477	210428	8437	3.28	36.50
6323	Reevesby Island	5776631	192029	8598	1.43	23.65
6320	Reevesby Island	5991492	189046	8589	1.54	25.09
6315*	Reevesby Island	5797846	201079	8589	1.54	22.50
N15NH10	Salutation Island	6028411	205372	8545	2.04	22.19
E2EB1	Salutation Island	6666027	232229	8640	0.95	22.38
W2WB1	Salutation Island	2175611	105402	7443	14.67	14.04
W1WG2	Salutation Island	5131050	195268	8508	2.46	20.53
N28NI1	Salutation Island	3025591	139815	7932	9.07	15.08
E5ED5	Salutation Island	3329310	153833	7966	8.68	15.59
W3WD1*	Salutation Island	3954834	166399	8395	3.76	17.85
E4EA5*	Salutation Island	2881333	142489	7824	10.31	14.47
E3ED6	Salutation Island	3841132	168735	8340	4.39	16.94
W8WE10	Salutation Island	6498047	215426	8603	1.38	23.80
W7WC19	Salutation Island	5176674	199259	8542	2.07	20.34
N6NC6	Salutation Island	3807970	164631	8240	5.54	17.10
W5WF9	Salutation Island	3479545	157681	8224	5.72	16.51
E7EF6	Salutation Island	3689540	166486	8219	5.78	16.17
N49NI8*	Salutation Island	4704528	188456	8316	4.67	18.49
E1EC6	Salutation Island	4478095	177991	8444	3.20	18.89
N45NE6	Salutation Island	5450092	205758	8382	3.91	19.85
N52NJ4	Salutation Island	2942568	135518	7827	10.27	14.91
N42ND8	Salutation Island	7170245	249368	8631	1.05	22.86
SNR07	St Peter Island	3706246	158380	8235	5.59	17.26

SNR04	St Peter Island	2396512	109850	7669	12.08	15.49
SNR10	St Peter Island	2753928	127082	7684	11.91	15.35
SNR11	St Peter Island	2928129	139151	7742	11.25	15.28
SNR13	St Peter Island	2894773	133032	7678	11.98	15.52
SNR12	St Peter Island	2501466	118638	7714	11.57	14.32
SNR08	St Peter Island	2695712	118689	7945	8.92	16.45
SNR02	St Peter Island	13861969	298900	8648	0.86	39.10
SNR03	St Peter Island	8946846	248072	8668	0.63	29.64
LC1414	West Franklin	2542062	121235	7608	12.78	13.84
LC1419	West Franklin	4581604	180037	8494	2.63	18.83
LC1428	West Franklin	5833920	209259	8607	1.33	21.70
LC1416	West Franklin	5682392	197233	8587	1.56	22.72
LC1437	West Franklin	4068915	150302	8298	4.87	19.55
LC1450	West Franklin	6616710	225660	8593	1.49	22.72
LC1417	West Franklin	4603828	178858	8482	2.76	19.10

SM Table 6. Average allelic error rates calculated for paired replicates, sub-sampled to different depths for the single nucleotide polymorphisms identified using ddRADseq.

		Number of Reads in Sub-sampled Replicate A		
		1 Million	**750k**	**500k**
Number of Reads Sub-sampled in Replicate B	**1 Million**	0.026	0.024	0.025
	750k	0.025	0.024	0.024
	500k	0.026	0.026	0.025

SM Table 7: Scaled root mean square error (SRMSE) for each sample size and statistic of down-sampled Reevesby Island individuals. Sample size (N), allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), individual inbreeding (individual F), effective population size estimated using the LD method (LD Ne) and effective population size estimated using the temporal method (Tp Ne).

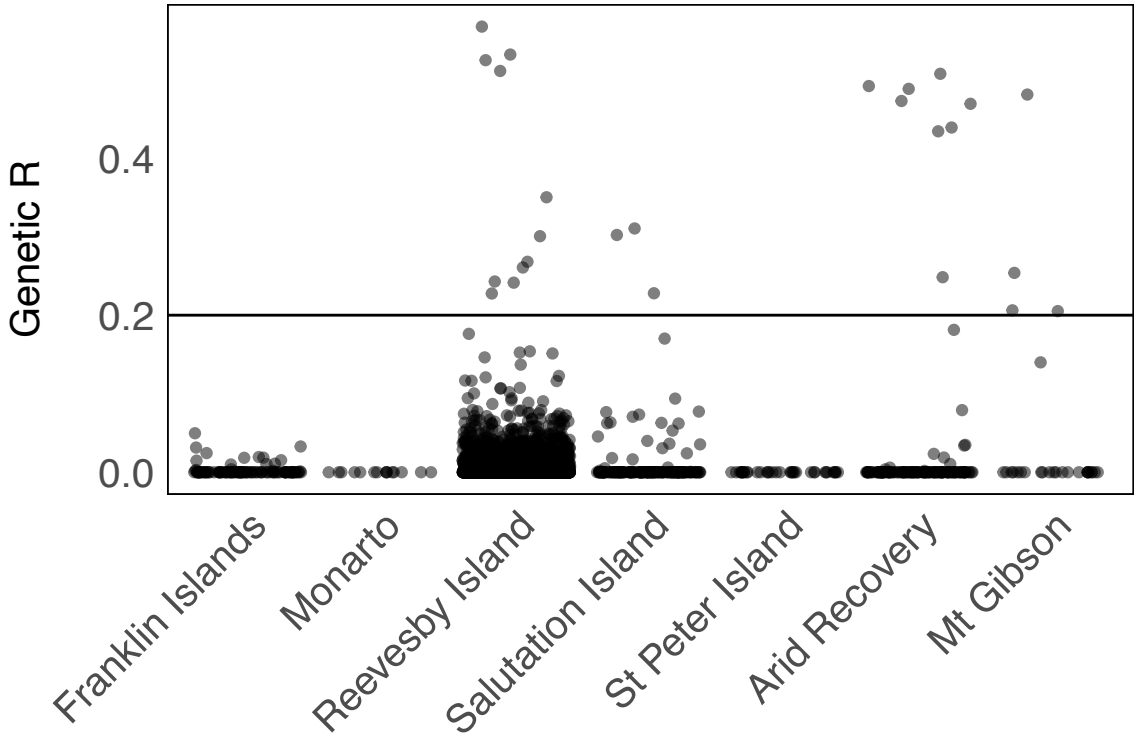
N	Ar	Ho	He	Individual F	LD Ne	Tp Ne
10	0.006	0.034	0.017	0.511	34.383	0.6
20	0.004	0.005	0.001	0.145	0.673	0.198
30	0.003	0.005	0.003	0.121	1.149	0.119
40	0.002	0.004	0.004	0.028	0.318	0.125

SI Table 8. Standard deviations of between-population genetic relatedness (genetic R).

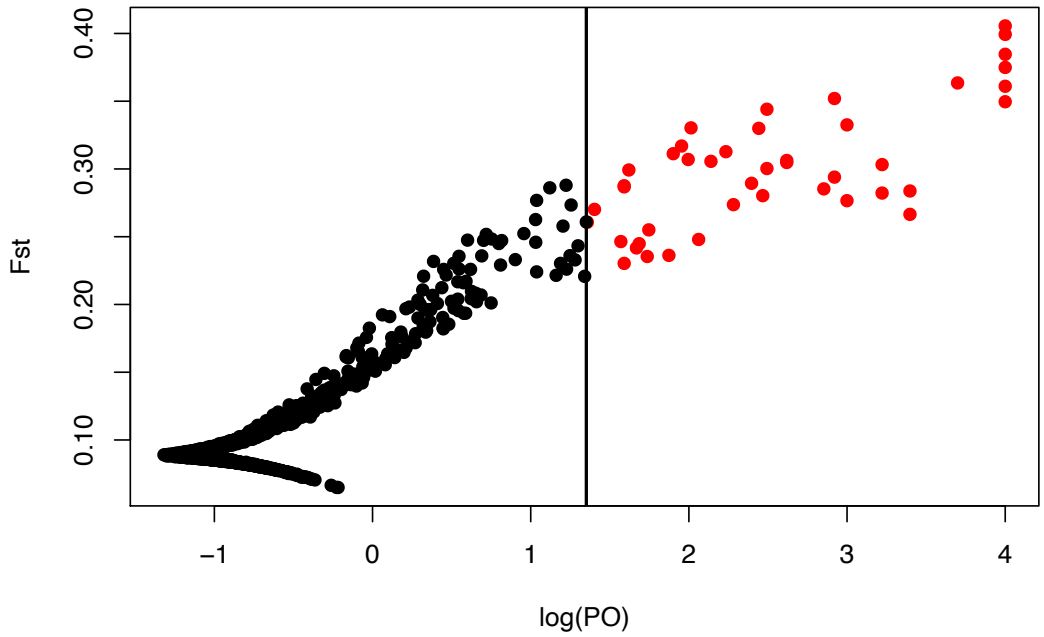
	Franklin Islands	West Franklin Island	East Franklin Island	Monarto	Reevesby Island	Salutation Island	St Peter Island	Arid Recovery	Mt Gibson
Franklin Islands	NA								
West Franklin Island	NA	NA							
East Franklin Island	NA	0.054	NA						
Monarto	0.101	0.038	0.121	NA					
Reevesby Island	0.045	0.037	0.049	0.063	NA				
Salutation Island	0.051	0.039	0.047	0.048	0.051	NA			
St Peter Island	0.042	0.045	0.039	0.062	0.046	0.051	NA		
Arid Recovery	0.040	0.038	0.042	0.053	0.048	0.039	0.039	NA	
Mt Gibson	0.048	0.030	0.038	0.043	0.051	0.044	0.050	0.064	NA

SI Table 9. 95% confidence intervals of pairwise F_{ST} results. Above the diagonal: upper CI, below the diagonal: lower CI.

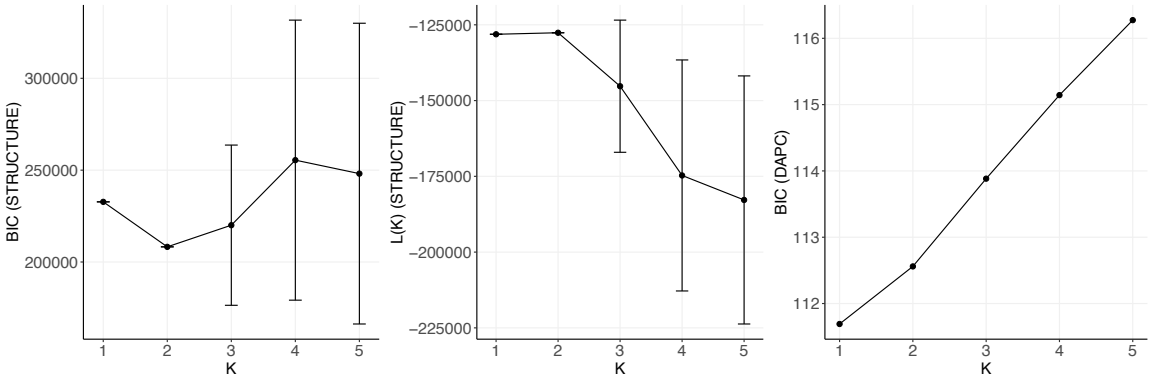
	Franklin Islands	West Franklin Island	East Franklin Island	Monarto	Reevesby Island	Salutation Island	St Peter Island	Arid Recovery	Mt Gibson
Franklin Islands	NA	NA	NA	0.014	0.037	0.114	0.045	0.069	0.096
West Franklin Island	NA	NA	0.041	0.045	0.042	0.12	0.056	0.08	0.103
East Franklin Island	NA	0.034	NA	0.01	0.053	0.143	0.06	0.087	0.132
Monarto	0.008	0.036	0.003	NA	0.03	0.141	0.032	0.061	0.145
Reevesby Island	0.033	0.037	0.048	0.024	NA	0.113	0.039	0.04	0.119
Salutation Island	0.106	0.11	0.133	0.129	0.107	NA	0.133	0.164	0.199
St Peter Island	0.039	0.048	0.052	0.025	0.034	0.123	NA	0.077	0.151
Arid Recovery	0.063	0.072	0.079	0.053	0.036	0.154	0.07	NA	0.163
Mt Gibson	0.088	0.093	0.121	0.133	0.109	0.186	0.139	0.151	NA



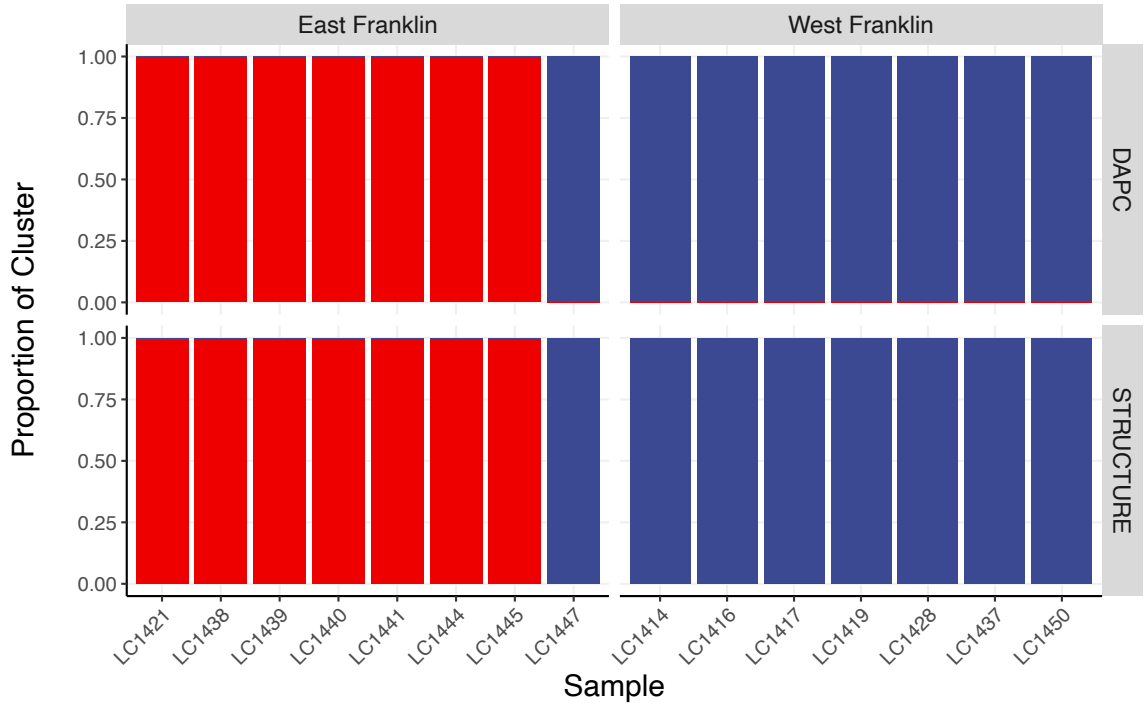
SM Fig. 1 Pairwise genetic relatedness for all pairs within each population of greater stick-nest rats. Genetic R was calculated for each population separately using the Hedrick and Lacy (2016) method in order to identify likely close relatives. Each dot represents a pair. The horizontal lines represents our cut-off of 0.2, above which pairs were considered first or second degree relatives.



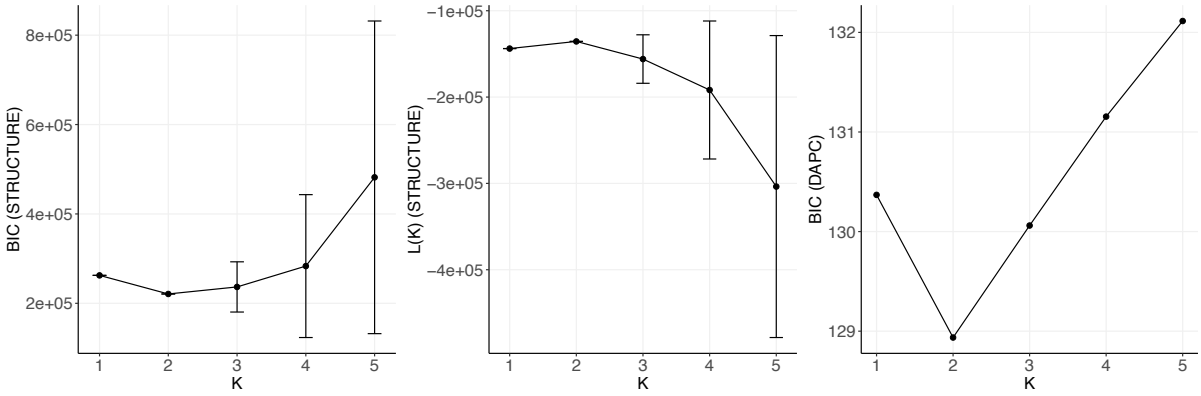
SM Fig. 2 Signatures of selection in the GSNR inferred using the program Bayescan. Each dot represents a locus. The vertical axis indicates mean FST between the seven populations and the horizontal axis indicates the log posterior odds (PO). The vertical line indicates the false discovery rate threshold of 0.01. Loci to the right of this line (red dots) are putatively under selection.



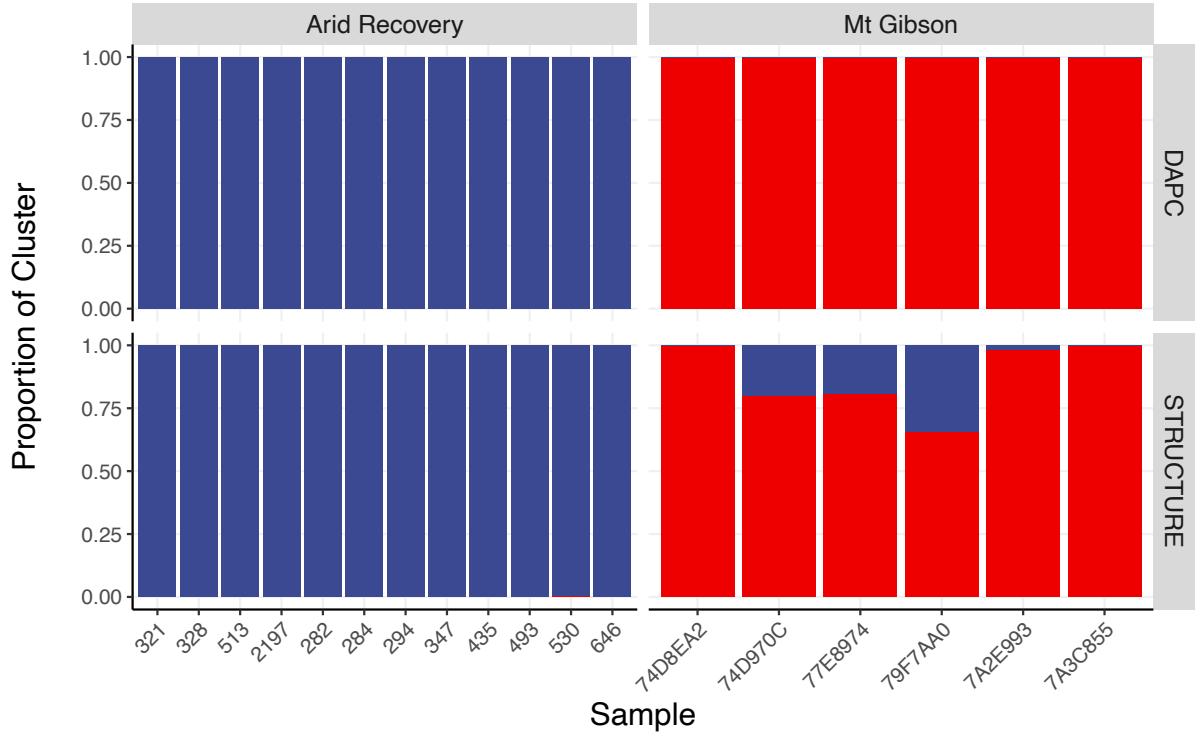
SM Fig. 3 Results from STRUCTURE and DAPC estimating the optimal K for the Franklin Island population. STRUCTURE finds the optimal K to be 2 (lowest BIC and highest L(K), while DAPC find the optimal K to be 1 (lowest BIC).



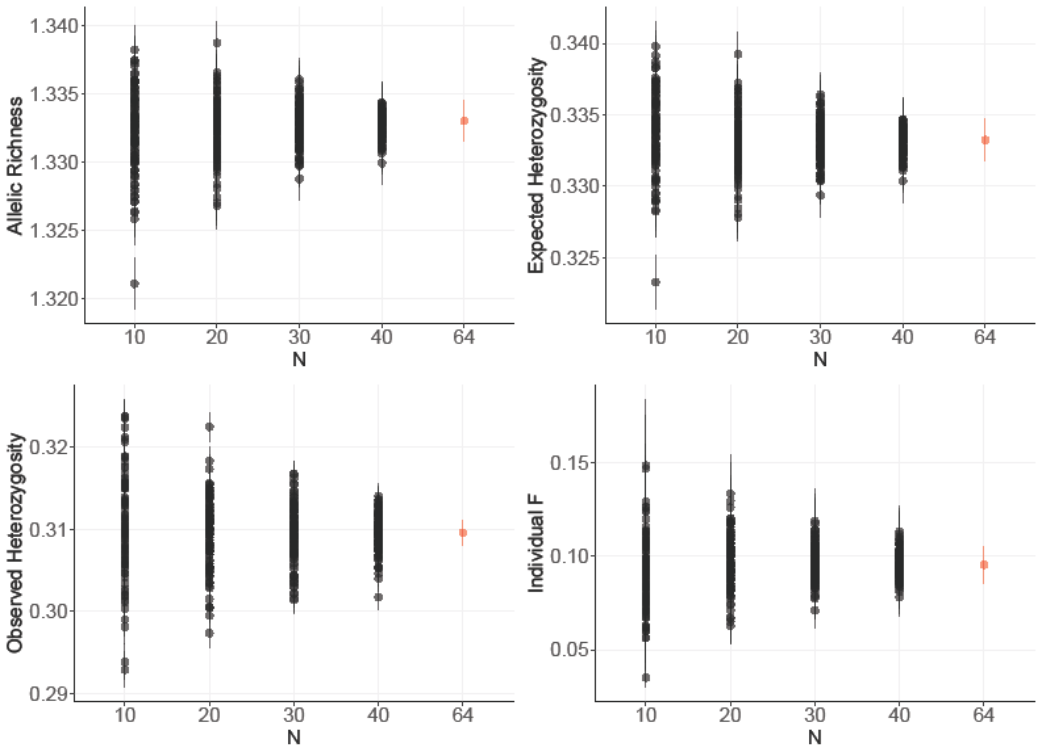
SM Fig. 4 Results of two K-means clustering methods (DAPC and STRUCTURE) for K = 2. Both methods found very little admixture of the ancestral groups within individuals, and both methods clustered one East Franklin Island individual (LC1447) with the West Franklin Island individuals.



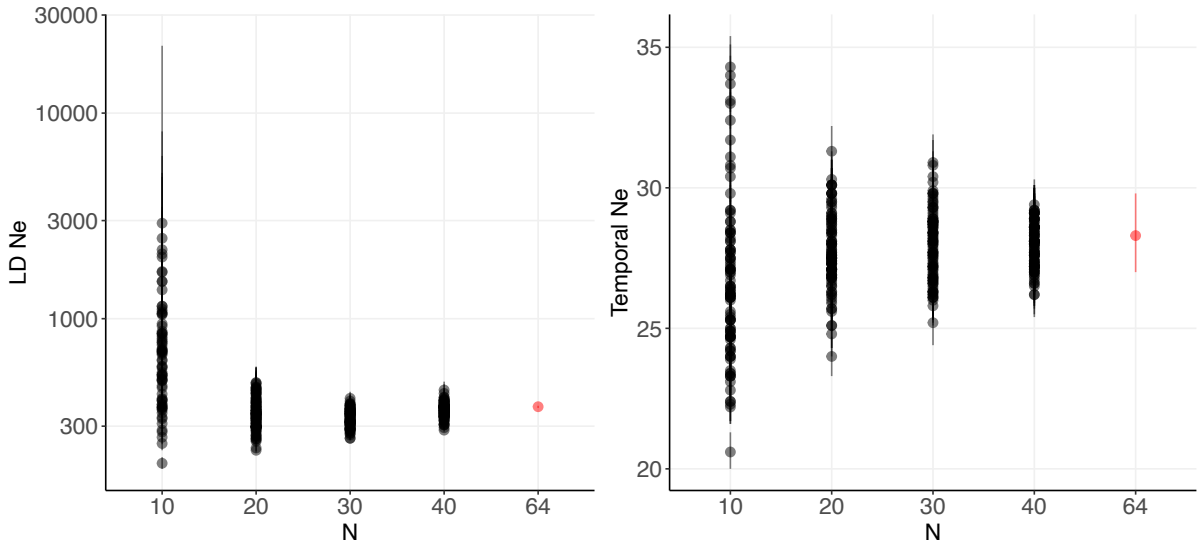
SM Fig. 5 Results from STRUCTURE and DAPC estimating the optimal K for the Arid Recovery and Mt Gibson individuals. Both methods find the optimal K to be 2 (lowest BIC and highest L(K).



SM Fig. 6 Results of two K-means clustering methods (DAPC and STRUCTURE) for K = 2 in the Arid Recovery and Mt Gibson individuals.



SM Fig. 7 Down-sampling results for diversity and inbreeding statistics. Individuals from Reevesby Island were down-sampled 100 times for each N (10, 20, 30, and 40) and summary statistics re-calculated to measure the robustness of each statistic to smaller sample sizes. Dots represent the mean value and lines represent standard errors. Black represents summary statistics calculated from down-sampled sample sets and red represents the statistics calculated from the full sample set (N = 64).



SM Fig. 8 Down-sampling results for Ne estimates. Individuals from Reevesby Island were down-sampled 100 times for each N (10, 20, 30, and 40) and Ne was re-estimated using both the temporal and LD method to measure the robustness of each method to smaller sample sizes. Dots represent the point estimates and lines represent standard errors. Black represents summary statistics calculated from down-sampled sample sets and red represents the estimate calculated from the full sample set (N = 64).