



Supplementary Information for

Cost, risk and avoidance of inbreeding in long-tailed tits

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This PDF file includes:

Supplementary text

Figure S1

Tables S1 to S10

Table S1. Cases of inbreeding in the Rivelin valley long-tailed tit population according to the social pedigree (1994-2016).

Pair ID	Male ID	Female ID	r_{QG}	r	Social relationship
2007/21	1945	1943	0.253	0.5	Full siblings (reared together)
2000/04	797	1080	0.219	0.25	Maternal $\frac{1}{2}$ siblings; male was cross-fostered
2011/15	2339	2408	0.212	0.25	Paternal uncle/niece
1996/19	698	633	0.273	0.125	Maternal $\frac{1}{2}$ aunt/nephew
2004/50	1516	1611	-0.151	0.125	Paternal cousins
2007/12	1761	2052	0.267	0.125	Paternal cousins

Relationships of $r \geq 0.125$ are considered inbred. Genetic relatedness estimates (r_{QG}) and social relationships are also reported.

Table S2. Cases of inbreeding in the Rivelin valley long-tailed tit population according to genetic estimates (1994-2016).

Pair ID	Male ID	Female ID	r_{QG}	r	Social relationship (if known)
2012/28	2283	468	0.571	0	
2003/27	265	266	0.452	0	
2008/28	435	458	0.372	0	
2004/06	290	264	0.351	0	
2015/21	3079	3185	0.347	0	
2005/01	290	356	0.332	0	
2014/05	3042	2947	0.327	0	
2007/71	446	2090	0.301	0	
2011/05	1978	520	0.297	0	
2015/11	3171	3170	0.283	0	
2004/37	294	242	0.282	0	
1996/19	698	633	0.273	0.125	Maternal ½ aunt/nephew
2016/19	3286	3285	0.269	0	
2015/08	2909	3182	0.267	0	
2007/12	1761	2052	0.267	0.125	Paternal cousins
2015/18	3179	2756	0.263	0	
2007/07	354	368	0.262	0	
2007/21	1945	1943	0.254	0.5	Full-sibs (reared together)
2007/69	448	390	0.25	0	
2012/51	2592	2762	0.244	0	
2006/32	1756	313	0.241	0	
2000/42	181	1098	0.229	0	
2016/17	3132	3074	0.226	0	Unrelated
2015/06	3092	3059	0.226	0	
2007/60	443	442	0.224	0	
2012/55	2712	2758	0.221	0	
1998/16	108	123	0.22	0	
2004/65	327	352	0.219	0	
2000/04	797	1080	0.219	0.25	Maternal ½ siblings; male was cross-fostered
2013/12	2779	2782	0.219	0	
2002/04	195	1192	0.217	0	
2003/09	1475	256	0.215	0	
2009/02	465	2124	0.215	0	
2011/04	2688	2687	0.213	0	
2011/15	2339	2408	0.212	0.25	Paternal uncle/niece
2014/08	2967	2782	0.213	0	
2002/30	1339	249	0.209	0	
2004/27	301	285	0.201	0	
2005/38	1589	368	0.198	0	
2009/17	2097	520	0.197	0	

2008/68	2137	496	0.195	0	
1998/11	848	819	0.195	0	unrelated
2015/05	2975	3055	0.193	0	
2005/35	1601	281	0.192	0	
2004/18	299	1591	0.191	0	
2013/29	2877	2916	0.184	0	
2007/55	2068	441	0.183	0	
2008/30	431	485	0.182	0	
1999/21	149	148	0.18	0	
2008/62	367	469	0.178	0	
2002/19	1367	207	0.176	0	
1999/26	166	140	0.174	0	
1998/22	136	46	0.174	0	
2011/07	3162	2693	0.172	0	
2016/04	3165	3274	0.172	0	
2007/14	404	324	0.169	0	
2007/39	505	504	0.168	0	
2011/41	2700	2701	0.167	0	
2012/13	2738	2739	0.166	0	
2012/62	2229	520	0.165	0	
2003/29	1347	242	0.163	0	
2002/12	229	228	0.161	0	
2001/25	214	1042	0.160	0	
2008/16	453	2060	0.159	0	
2005/31	1602	363	0.157	0	
2008/21	2050	463	0.157	0	
2005/08	1689	323	0.157	0	
2016/05	3206	3276	0.155	0	
2006/22	354	270	0.155	0	
2008/13	381	1941	0.153	0	
2013/15	2723	2722	0.153	0	
2008/20	1930	1402	0.151	0	unrelated
2009/01	367	2090	0.151	0	
2003/24	1349	217	0.149	0	
2004/16	303	302	0.147	0	
2009/27	2159	483	0.147	0	
2012/12	2740	2695	0.146	0	
2004/54	308	320	0.146	0	
2005/47	1739	377	0.146	0	
2010/09	2381	2380	0.145	0	
2016/26	3087	3061	0.145	0	
2011/25	2717	2718	0.143	0	
2000/29	1071	169	0.141	0	
2010/43	429	520	0.141	0	
2012/01	2714	2705	0.14	0	

2014/03	3007	3056	0.138	0	
2006/48	1512	350	0.137	0	
2008/50	476	270	0.133	0	
1999/30	965	863	0.133	0	unrelated
1997/13	108	107	0.132	0	
2014/37	2907	3009	0.132	0	
2006/10	386	356	0.132	0	
2004/39	1641	332	0.131	0	
2007/42	429	430	0.126	0	
1997/31	671	103	0.126	0	
2001/01	201	200	0.125	0	

Relationships of $r_{QG} \geq 0.125$ are considered inbred. Pedigree relatedness (r) and social relationships are also reported.

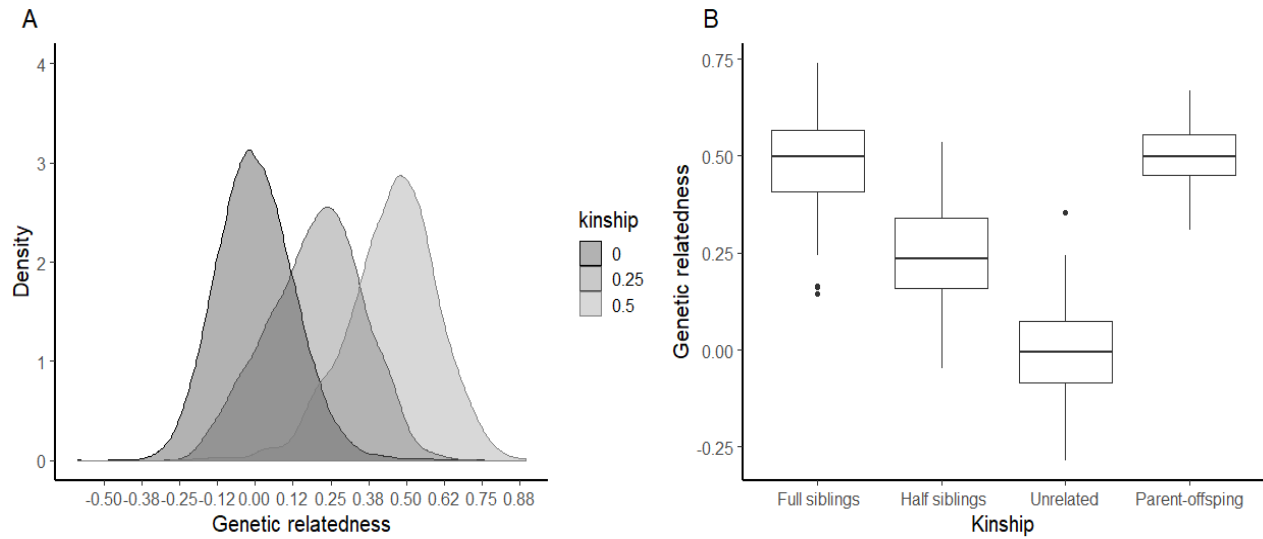


Figure S1. (a) The distribution of genetic relatedness estimates (r_{QG}) among known first-order (kinship = 0.5), second-order (kinship = 0.25) and non-kin (kinship = 0) from the long-tailed tit social pedigree; (b) box plot of genetic relatedness estimates (r_{QG}) for simulated individuals of known relatedness, using allele frequencies from the genetic data.

Table S3. Model outputs from a GLMM testing for an association between heterozygosity (H) at microsatellite loci and probability of recruitment.

<i>AIC</i>	<i>BIC</i>	<i>logLik</i>	<i>deviance</i>	<i>residual Df</i>	<i>N</i>
1656.2	1689.6	-822.1	1644.2	1918	1924

<i>Fixed effects</i>	<i>Estimate</i> <i>SE</i>	<i>Z</i>	<i>p</i>	<i>Random effect</i>	<i>Variance ± SD</i>
(Intercept)	-1.863 ± 1.12	-4.925	<0.001	Brood (<i>N</i> = 257)	1.519 ± 1.23
H	0.233 ± 0.57	0.404	0.686		
Helper number	0.301 ± 0.08	3.716	< 0.001		
Sex	0.996 ± 0.14	6.918	< 0.001		
Fledge date	-0.014 ± 0.01	-1.177	0.239		

Table S4. Model outputs from a GLMM testing for an association between heterozygosity (H) at microsatellite loci and hatching success in females.

<i>AIC</i>	<i>BIC</i>	<i>logLik</i>	<i>deviance</i>	<i>residual Df</i>	<i>N</i>
121.5	130.9	-54.8	109.5	29	35
<i>Fixed effects</i>	<i>Estimate</i> <i>SE</i>	<i>± Z</i>	<i>p</i>	<i>Random effect</i>	<i>Variance ± SD</i>
(Intercept)	0.780 ± 4.42	0.177	0.86	Brood (<i>N</i> = 31)	0.836 ± 0.914
H	5.052 ± 2.17	2.326	0.02	Year (<i>N</i> = 13)	< 0.001
Mass at 11 days old	-0.022 ± 0.02	-1.020	0.31		
Lay date	-0.42 ± 0.45	-0.930	0.35		

Table S5. Model outputs from a GLMM testing for an association between heterozygosity (H) at microsatellite loci and probability of producing recruits.

<i>AIC</i>	<i>BIC</i>	<i>logLik</i>	<i>deviance</i>	<i>residual Df</i>	<i>N</i>
124.7	134.6	-58.4	116.7	83	87
<i>Fixed effects</i>	<i>Estimate</i> <i>SE</i>	<i>± Z</i>	<i>p</i>	<i>Random effect</i>	<i>Variance ± SD</i>
(Intercept)	2.849 ± 1.73	1.643	0.1004	Brood (N = 58)	< 0.001
H	-2.966 ± 1.67	-1.769	0.077		
Male fledglings	0.079 ± 0.53	0.148	0.882		

Table S6. Model outputs from a GLMM testing for an association between heterozygosity (H) at microsatellite loci and direct fitness.

<i>AIC</i>	<i>BIC</i>	<i>logLik</i>	<i>deviance</i>	<i>residual Df</i>	<i>N</i>
35.4	45.6	-11.7	23.4	34	40

<i>Fixed effects</i>	<i>Estimate</i> <i>SE</i>	<i>±</i>	<i>t</i>	<i>p</i>	<i>Random effect</i>	<i>Variance ± SD</i>
(Intercept)	3.281 ± 0.62		5.277	< 0.001	Brood (N = 32)	0.634 ± 0.79
H	-2.741 ± 0.58		-4.654	< 0.001	Residual	0.141 ± 0.37
Sex	0.446 ± 0.15		2.824	< 0.01		
Male fledglings	1.80 ± 0.44		4.057	< 0.001		

Table S7. Effect estimates from LMMs comparing the observed genetic relatedness of females' chosen partner to that expected under mate choice models.

Range (m)	<i>N</i>	Mate choice model	Estimate ± SE	df	<i>t</i>	<i>P</i>
300	2420	Pair relatedness (<i>intercept</i>)	0.002 ± 0.004	771	0.47	0.64
		Random mate choice	0.03 ± 0.004	1801	7.23	< 0.001
		Close kin avoided	0.001 ± 0.004	1801	0.36	0.72
		Close & distant kin avoided	-0.04 ± 0.004	1804	-9.9	< 0.001
600	2433	Pair relatedness (<i>intercept</i>)	0.002 ± 0.004	1009	0.6	0.55
		Random mate choice	0.01 ± 0.004	1817	3.93	< 0.001
		Close kin avoided	-0.005 ± 0.004	1817	-1.32	0.18
		Close & distant kin avoided	-0.04 ± 0.004	1817	-11.46	< 0.001
900	2433	Pair relatedness (<i>intercept</i>)	0.002 ± 0.003	1110	0.67	0.5
		Random mate choice	0.01 ± 0.003	1815	3.03	< 0.01
		Close kin avoided	-0.004 ± 0.003	1815	-1.15	0.25
		Close & distant kin avoided	-0.04 ± 0.003	1816	-11.52	< 0.001
1200	2433	Pair relatedness (<i>intercept</i>)	0.002 ± 0.003	1129	0.69	0.49
		Random mate choice	0.006 ± 0.003	1814	1.9	0.06
		Close kin avoided	-0.005 ± 0.004	1814	-1.46	0.14
		Close & distant kin avoided	-0.04 ± 0.004	1815	-11.8	< 0.001

Mate choice models assume (i) random mate choice, (ii) avoidance of close kin and (iii) avoidance of close and distant kin, when mates are selected from within multiple pairing ranges. In each model, female identity nested within year was fitted as a random effect (variance < 0.01, *N* = 609).

Table S8. Effect estimates from LMMs comparing the observed genetic relatedness of males' chosen partner to that expected under mate choice models.

Range (m)	<i>N</i>	Mate choice model	Estimate ± SE	df	<i>t</i>	<i>P</i>
300	2416	Pair relatedness (<i>intercept</i>)	0.002 ± 0.004	634	0.67	0.5
		Random mate choice	0.02 ± 0.004	1794	7.84	< 0.001
		Close kin avoided	0.005 ± 0.004	1794	1.47	0.14
		Close & distant kin avoided	-0.03 ± 0.004	1797	-9.16	< 0.001
600	2432	Pair relatedness (<i>intercept</i>)	0.003 ± 0.004	858	0.85	0.39
		Random mate choice	0.02 ± 0.003	1813	5.14	< 0.001
		Close kin avoided	-0.001 ± 0.003	1813	-0.05	0.96
		Close & distant kin avoided	-0.04 ± 0.003	1814	-11.04	< 0.001
900	2432	Pair relatedness (<i>intercept</i>)	0.003 ± 0.003	986	0.79	0.43
		Random mate choice	0.01 ± 0.003	1810	3.79	< 0.001
		Close kin avoided	-0.001 ± 0.003	1810	-0.29	0.77
		Close & distant kin avoided	-0.04 ± 0.003	1811	-11.19	< 0.001
1200	2432	Pair relatedness (<i>intercept</i>)	0.002 ± 0.003	1062	0.75	0.45
		Random mate choice	0.009 ± 0.003	1808	2.54	< 0.05
		Close kin avoided	-0.003 ± 0.003	1808	-0.84	0.39
		Close & distant kin avoided	-0.04 ± 0.003	1809	-11.54	< 0.001

Mate choice models assume (i) random mate choice, (ii) avoidance of close kin and (iii) avoidance of close and distant kin, when mates are selected from within multiple pairing ranges. In each model, male identity nested within year was fitted as a random effect (variance < 0.01, *N* = 609).

Table S9. Effect estimates from a LMM comparing the dissimilarity of churr calls among opposite-sex long-tailed tit dyads of varying kinship.

logLik	Residual df	Kinship	Estimate \pm SE	<i>t</i>	<i>p</i>
1800	1341	First-order kin (<i>intercept</i>)	4.69 \pm 0.31	15.01	< 0.001
		Second-order kin	-0.74 \pm 0.25	-3.02	0.002
		Non-kin	-0.88 \pm 0.25	-3.62	< 0.001

Degrees of kinship: first-order kin ($N = 20$); second-order kin ($N = 249$); non-kin ($N = 1078$).

Call dissimilarity was measured using dynamic time warping analysis (DTW) and modelled as a continuous response with Gaussian distribution and log link. Kinship was fitted as a fixed effect, with male ID (variance = 0.07 ± 0.28 , $N = 46$) and female ID (variance \pm SD = 0.16 ± 0.40 , $N = 41$) fitted as random effects.

Table S10. Effect estimates from a LMM comparing the dissimilarity of churr calls among four groups of opposite-sex long-tailed tit dyads within pairing range ($\leq 1350\text{m}$).

logLik	Residual df	Group	Estimate \pm SE	<i>t</i>	<i>p</i>
1253	945	Breeding pairs (<i>intercept</i>)	4.00 \pm 0.24	16.35	< 0.001
		First-order kin	1.11 \pm 0.39	2.87	0.004
		Second-order kin	0.01 \pm 0.12	0.06	0.94
		Non-kin	-0.16 \pm 0.10	-1.63	0.10

Comparison groups: breeding pairs ($N = 51$); potential first-order kin pairings ($N = 11$); potential second-order kin pairings ($N = 155$); potential non-kin pairings ($N = 735$). Call dissimilarity was measured using dynamic time warping analysis (DTW) and modelled as a continuous response with Gaussian distribution and log link. 'Group' was fitted as a fixed effect, with male ID (variance \pm SD = 0.11 \pm 0.34, $N = 48$) and female ID (variance \pm SD = 0.25 \pm 0.51, $N = 45$) fitted as random effects.