Supplementary information

Dynamics and genetics of a disease-driven species decline to near extinction: lessons for conservation.

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Supplementary Table 1. Mountain chickens found dead or with severe signs of chytridiomycosis on Dominica between 2003-2004

Date	Site	Number dead	Number with signs of chytridiomycosis
13/12/2002	Gallion	2	0
13/01/2003	Bagatelle	0	1
24/01/2003	Galion	0	2
30/01/2003	Elmshall	1	0
01/02/2003	La Haut	1	0
17/02/2003	Bois Cotelette	1	0
19/02/2003	Petit Coulibri	1	1
25/02/2003	Petit Coulibri	0	1
25/02/2003	Soufriere	1	1
06/03/2003	Dublanc Valley	1	0
19/03/2003	Bagatelle	0	1
26/03/2003	Bois Cotelette	0	1
28/03/2003	Dublanc Valley	3	1
01/04/2003	La Haut	4	0
26/12/2003	Milton Estate	1	0
17/01/2004	Elmshall	1	0
12/02/2004	Upper Kings Hill	1	0
14/04/2004	Coulibistre	1	0
15/06/2003	Fond St. Jean	2	4
26/06/2003	Fond St. Jean	1	5

Supplementary Table 2. Summary statistics of all loci in all populations

NA = Number of alleles, H_O = Observed heterozygosity, H_E = Expected heterozygosity. Loci significantly (p=<0.05) deviating from HWE indicated in shaded cell, red = heterozygote deficiency, green = heterozygote excess. Significant (p= <0.05) F_{IS} values are shown in bold.

	Locus	NA	H_O	H_E	F_{IS}
Founders	0673_p1	3	0.545	0.450	-0.224
	759A_p1	6	0.818	0.688	-0.200
	0867_p1	3	0.545	0.437	-0.263
	2969_p1	3	0.727	0.662	-0.103
	3035_p1	2	0.364	0.485	0.259
	1628_p1	3	0.000	0.606	**1.000**
	3956_p1	3	0.182	0.589	**0.701**
	7957_p1	5	0.909	0.684	-0.351
Wild	0673_p1	4	0.473	0.441	-0.072
	759A_p1	6	0.838	0.724	**-0.158**
	0867_p1	6	0.581	0.528	-0.101
	2969_p1	3	0.622	0.650	0.044
	3035_p1	3	0.500	0.529	0.055
	1628_p1	3	0.041	0.588	**0.931**
	3956_p1	4	0.081	0.516	**0.844**
	7957_p1	6	0.740	0.741	0.001
Post Dom	0673_p1	3	0.529	0.558	0.053
	759A_p1	6	0.294	0.627	**0.539**
	0867_p1	5	0.412	0.592	0.311
	2969_p1	3	0.353	0.426	0.176
	3035_p1	3	0.235	0.314	0.256
	1628_p1	2	0.353	0.471	0.256
	3956_p1	2	0.000	0.148	**1.000**
	7957_p1	4	0.647	0.770	0.164
Mont	0673_p1	4	0.550	0.514	-0.071
	759A_p1	8	0.825	0.745	**-0.108**
	0867_p1	6	0.533	0.501	-0.064
	2969_p1	3	0.622	0.656	0.052
	3035_p1	4	0.492	0.506	0.029
	1628_p1	3	0.025	0.571	**0.956**
	3956_p1	4	0.100	0.477	**0.791**
	7957_p1	6	0.765	0.722	-0.059
Pre Dom	0673_p1	7	0.621	0.743	0.168
	759A_p1	8	0.759	0.736	-0.031
	0867_p1	10	0.759	0.814	0.069
	2969_p1	5	0.370	0.387	0.044
	3035_p1	4	0.357	0.611	**0.420**
	1628_p1	4	0.138	0.356	**0.616**
	3956_p1	5	0.148	0.546	**0.733**
	7957_p1	4	0.793	0.702	-0.132
Dom	0673_p1	7	0.596154	0.677931	0.122
	759A_p1	8	0.615385	0.710232	0.135
	0867_p1	10	0.634615	0.744586	**0.149**
	2969_p1	5	0.36	0.391919	0.082
	3035_p1	5	0.313725	0.558144	**0.44**
	1628_p1	4	0.230769	0.434653	**0.472**
	3956_p1	5	0.088889	0.453433	**0.806**
	7957_p1	4	0.769231	0.720127	-0.069

Supplementary data 1. Mitochondrial DNA sequences

A 463bp fragment of the cytochrome oxidase subunit I gene was amplified for 32 individuals (16 randomly chosen samples from each island) using universal amphibian primers developed by Che *et al.* (2012): Chmf4, 5' - TYT CWA CWA AYC AYA AAG AYA TCG G – 3';Chmr4, 5' - ACY TCR GGR TGR CCR AAR AAT CA - 3'. To produce a final PCR reaction volume of 10µl, the following reagents were included, 5µl Qiagen Multiplex mix, 0.6µl of 0.1pmol/µl reverse and forward primer, 1µl of DNA and 2.8µl of nuclease free ddH_2O. The PCR conditions were as follows: 95^oC for 15min, followed by 35 cycles of: 94^oC for 1min, 56^oC for 1min, extension for 1min at 72^oC; and a final extension of 10min at 72^oC. Un-purified PCR products were sequenced in both directions using the Eurofins MWG Operon's DNA sequencing service. Sequences were verified using Sequencher ® (version 5.1) and subsequently aligned by the ClustalW multiple sequence alignment algorithm (Thompson *et al.,* 2002) implemented in BioEdit Sequence Alignment Editor (Hall, 1999; version 7.1.11) with the default parameters. A single haplotype was present in all individuals (GenBank accession number KX579492).

Che, J., H.-M. Chen, J.-X. Yang, J.-Q. Jin, K. Jiang, Z.-Y. Yuan, R. W. Murphy, and Y.-P. Zhang. 2012. Universal COI primers for DNA barcoding amphibians. *Molecular Ecology Resources* 12:247–58.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposium series* 41: 95-98).

Sequencher® version 5.4.5 sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com

Thompson, J.D., Gibson, T. and Higgins, D.G., 2002. Multiple sequence alignment using ClustalW and ClustalX. *Current protocols in bioinformatics,* pp.2-3.

Supplementary data 2. Definitions of extent of occurrence (EOO) and area of occupancy (AOO)

The EOO is defined as “The area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon” (IUCN, 2001). This was calculated by drawing a minimum convex polygon around the midpoints of all transects on which mountain chickens had been observed. This likely represented an overestimate as it almost certainly included areas of unsuitable habitat. The AOO is defined as “The area within its 'extent of occurrence', which is occupied by a taxon, excluding cases of vagrancy” (IUCN, 2001). This was calculated by overlaying a 500 m grid on both islands and assuming full occupancy of a grid square if mountain chicken presence had been confirmed on a transect which had its midpoint within a grid square. A 500 m grid size was chosen as it represents the best current guess of the home range size of the mountain chicken (authors’ unpublished data).

Reference:

IUCN (International Union for the Conservation of Nature), 2001. IUCN Red List Categories and Criteria version 3.1.

Supplementary data 3. Microsatellite primer development

Microsatellite primers for the mountain chicken were isolated commercially by Ecogenics (Switzerland). Eight polymorphic markers (Supplementary Table 3) were then standardised using a Qiagen Multiplex PCR Kit using the following reaction mix: 5 µl of Multiplex mix, 0.2ul of 0.2 pmol/µl

of forward primer, 0.2µl of 0.2pmol/µl of reverse primer, 1µl of DNA and 3.6µl of nuclease free ddH_2O. PCR conditions were as follows: initial denaturation at 95^oC for 15min, followed by 35 cycles of: 30s at 94^oC, 90s at specific annealing temperature (Supplementary Table 3), extension for 60s at 72^oC; and a final extension of 30min at 72^oC. All genotyping was performed at DNA Sequencing and Services (Dundee) and microsatellite scoring was performed using GeneMapper (v4).

The microsatellite genotypes will be submitted to Dryad upon acceptance of this manuscript for publication, and the corresponding accession code will be provided here.

Supplementary Table 3. Polymorphic microsatellites identified for *Leptodactylus fallax*

For each marker the annealing temperature (Tm ^oC), number of observed alleles (NOA), repeat motif (mot), PCR products allele size range in base pairs (range), combinations of primers for multiplexing (M) are shown.

Primer Code	Tm (°C)	Primer sequence (5'-3')	NOA	Mot	Range	M
Lepfal_010673	62	AGCAATTCTTGTTGCCTCCC	5	(TAGA)	217-241	3
		AGCCTAAGTTCTTGCAGGGC				
Lepfal_015759A	64	AAGATCAGCCAGGGACAGAC	10	(TTTC)	189-234	3
		CACTGTGATATTTAGGGGTGC				
Lepfal_000867	64	CGTGAGAAAGACTAGGGCAC	10	(TAGA)	200-244	1
		AAAAGGGAGCACTCCACAGG				
Lepfal_002969	60	AGCATCACAGGGAACCAGTC	5	(AC)	169-191	2
		GCTCCTGAAGTACAAACGCC				
Lepfal_003035	62	ACATACAGAACTGCTTACATGTCC	5	(TG)	126-134	1
		GCTTTGTCACTGGCTCCAAG				
Lepfal_011628	60	ATGATTGGCCCCAGTGTATG	4	(CA)	207-234	2
		GATCGCAGAACCTGGACCTC				
Lepfal_013956	58	AGCGTTCGATTAGTAGCTGTG	5	(AC)	134-162	2
		AGTTCACCCCAACGTAGGAC				
Lepfal_017957	58	TGTATGATGTGGGCCTTCCC	6	(TG)	189-242	1
		CACCACTGAAATAACCTATCATTTGTC				