The following supplement accompanies the article

Evaluating a potential source of founders for *ex situ* conservation efforts: genetic differentiation between disjunct populations of the endangered red siskin *Spinus cucullatus*

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Supplementary Materials

Detailed methods

DNA Amplification and sequencing – For cytochrome B, our PCR reactions included 1x PCR buffer, 1.5mM $MgCl_2$, 0.2mM of each dNTP, 0.8uM of each primer, 1% DMSO, 1 unit Biolase Taq, and ~25ng template DNA in a total volume of 25ul. The PCR thermal profile began with preheating at 95^{o}C for 3 min, followed by 35 cycles of denaturing at 95^{o}C for 30 seconds, annealing at 50^{o}C for 30 sec and extending at 72^{o}C for 1 min. The final extension step lasted 10 min.

For the control region, we adjusted PCR components to include 1x PCR buffer, 2.0 mM $MgCl_2$, 0.05mM of each dNTP, 0.4 uM of each primer, 8x bovine serum albumin, 1 unit Biolase Taq, and ~100ng template DNA, in a total volume of 25ul. Our final PCR thermal profile for this gene included a 'touch-down' annealing temperature to ensure specific primer-template binding, as follows: preheating at 95^{o}C for 10 min, followed by 2 cycles of denaturing at 95^{o}C for 15 seconds, annealing at 60^{o}C for 30 sec and extending at 72^{o}C for 1 min, with subsequent pairs of cycles the same except stepping down the annealing temperature to 58, 56, 54, and 52 ^{o}C. The final 35 cycles used an annealing temperature of 50^{o}C, and the final extension step lasted 10 min.

PCR products were purified by incubating 12ul product with 6ul ultrapure H_2O and 2ul ExoSap-IT (Affymetrix) at 37^{o}C for 30 min, and then at 80^{o}C for 15 min prior to cycle sequencing using 'Big Dye'(Applied Biosystems Inc). Labelled fragments were purified using Sephadex (GE Healthcare Life Sciences) according to manufacturer instructions, prior to drying and resuspending in formamide for sequencing. All amplicons were sequenced at least once (and often more than once) in each direction for each gene on an ABI PRISM 3100 genetic analyzer, and were aligned with Sequencher 5.0 (GeneCodes) to arrive at a consensus sequence for each gene from each individual. We used a BLAST search in GenBank to ensure that the resulting sequences were similar to sequences from related taxa.

AFLP amplification -- Amplified fragment-length polymorphisms (AFLP) are a class of markers that, although dominant and biallelic, are reliable, rapid and inexpensive to develop in large numbers in non-model organisms. Thus AFLP are ideally suited to a situation in which limited samples make the development of more expensive or labor-intensive markers difficult to justify. We used the protocol, AFLP adapters, and pre-selective primers described in Kingston et al. (2004) to screen all 18 individuals for variation with 14 selective primer pair combinations (Table S1. Two additional combinations were tested but not used due to poor amplification; data not shown). Our modifications to the protocol

included the following: we added bovine serum albumin at the initial Taq1 digestion stage rather than the initial EcoR1 digestion; we omitted this reagent from pre-selective and selective PCR steps (though we retained other modifications developed for degraded template); we diluted pre-selective PCR products into sterile water rather than TE 0.1 pH 8.0; and we used Biolase Taq (Bioline) in both PCR steps.

To detect fragment sizes, we used an ABI Prism 3100 genetic analyzer with a 96-capillary array, and loaded 1.5ul of ROX-labeled 78-559 size standard, with 13ul formamide (Applied Biosystems) and two selective PCR products, each produced with the same Taq 1 primer but different Eco R1 primers labeled with different dyes (Table S1; e.g., fragments amplified with primer pair 01 were co-loaded with those from primer pair 09). We co-loaded 2.5ul of all 6-FAM-labeled selective PCR products with 3.0ul of TET-labeled products, in order to bring blue and green fluorescence levels into similar ranges.

Table S1: Fourteen selective primer pair combinations used to develop AFLP loci.

	Taq* 1 trinucleotide extension						
***Eco* R1 trinucleotide extension*, dye**	**AAC**	**AAG**	**ACA**	**ACT**	**AGA**	**AGT**	**ATC**
AAC, 6-FAM	01	02	03	04	05	06	07
AAG, TET	09	10	11	12	13	14	15

*Extensions were attached, respectively, to the 3' end of the fluorescently labeled Eco R1 primers—5'-GACTGCGTACCAATTC–NNN-3' and the unlabeled Taq 1 primer—5'-GATGAGTCCTGACCGA–NNN-3'.

Supplementary Results

Table S2a: Raw simulation MLLOD scores for simulations in which Guyana genotypes were simulated to be full sibs (SIMGU) or Venezuela genotypes were simulated to be half sibs (SIMVE).

Simulation number	MLLOD	
	SIMGU	SIMVE
1	17.284	26.251
2	21.412	34.716
3	21.246	29.910
4	15.758	25.505
5	23.802	22.876
6	23.403	24.134
7	21.725	19.448
8	25.728	28.972
9	26.984	23.463
10	24.149	23.515
11	34.451	25.278
12	25.228	34.309
13	31.547	27.114
14	25.387	26.619
15	21.318	29.789
16	16.105	25.205
17	18.400	22.236
18	17.413	26.320
19	19.487	25.111
20	21.174	25.967
21	17.785	22.589
22	15.067	26.132
23	23.145	14.630
24	22.834	26.622
25	23.773	26.087
26	23.968	31.569
27	19.027	31.722
28	28.083	32.664
29	23.568	27.754
30	16.408	26.195
31	16.870	26.675
32	24.286	25.589
33	13.129	27.564
34	23.021	28.071
35	19.810	28.047
36	23.274	25.504
37	17.798	25.839
38	20.176	28.884
39	20.639	31.781
40	21.870	22.665
41	18.177	23.592
42	25.699	24.112
43	24.542	29.730
44	20.864	26.603
45	19.960	32.406
46	15.152	26.126
47	27.639	25.236
48	22.671	25.384
49	22.868	25.499
50	20.337	23.524
51	18.932	25.966
52	19.781	21.928
53	23.300	30.653
54	23.200	27.258
55	17.203	30.783
56	25.486	23.462
57	14.679	25.705
58	20.281	32.175
59	23.546	22.862
60	19.456	28.879
61	23.075	27.281
62	29.757	22.983
63	21.638	24.518
64	20.346	32.215
65	30.346	29.668
66	15.070	27.273
67	19.666	20.454
68	24.358	25.601
69	22.995	24.601
70	23.767	28.523
71	16.875	21.102
72	20.281	27.734
73	24.250	27.773
74	18.190	21.256
75	26.607	26.767
76	22.098	25.060
77	18.731	27.219
78	21.280	29.643
79	28.850	31.580
80	25.602	24.504
81	24.895	26.185
82	21.892	27.246

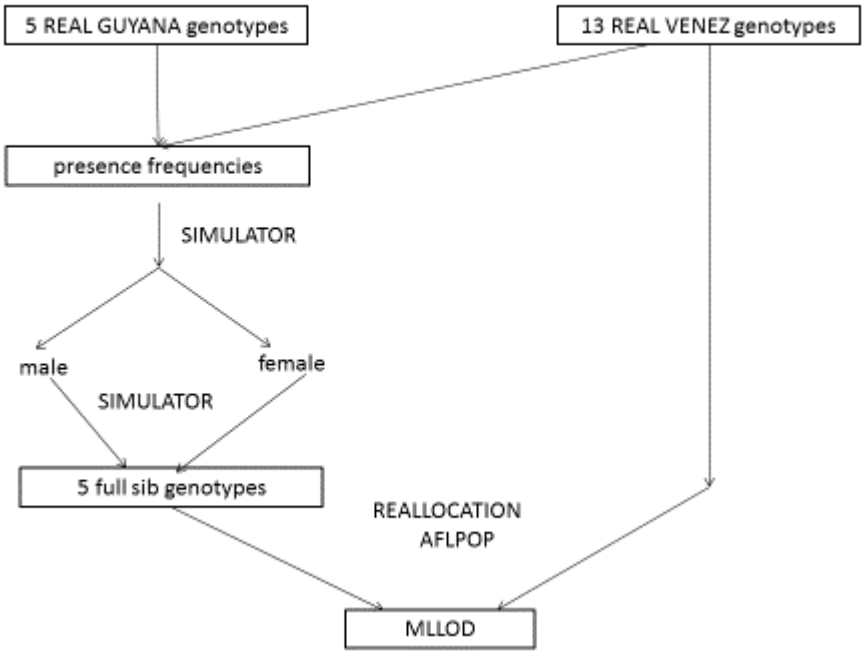
Simulation number	MLLOD	
	SIMGU	SIMVE
83	27.710	31.241
84	16.414	28.957
85	22.376	27.357
86	18.153	24.332
87	31.603	22.165
88	27.428	26.358
89	28.602	32.360
90	26.162	24.847
91	26.342	29.242
92	18.936	22.519
93	18.536	23.662
94	28.575	30.669
95	19.214	27.514
96	19.696	28.656
97	21.858	28.287
98	16.810	27.995
99	20.294	26.447
100	20.179	26.573

Table S2b: Summary statistics for MMLOD scores for simulations in which Guyana genotypes were simulated to be full sibs (SIMGU) or Venezuela genotypes were simulated to be half sibs (SIMVE).

Statistic	SIMGU	SIMVE
Mean	21.998	26.636
Standard deviation (SD)	4.179	3.365
Maximum	34.451	34.716
Minimum	13.125	14.630
Real MLLOD	37.797	37.797
Real MLLOD - Mean (in SD)	3.781	3.317
p-value	**<0.01**	**<0.01**

Figure S1: Simulation flow chart, showing the sequence of computation in one simulation for scenarios in which a) GU genotypes are replaced with the simulated genotypes of 5 full sibs and b) VE genotypes are replaced with the simulated genotypes of 13 half sibs.

a)



b)