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Accessory Publication

Methods

Simulations to test ability of our markers to detect genetic structure

In order to determine the conditions under which lack of gene flow would be detected, we used the approach of Lada *et al*. (2008a) of running simulations in EASYPOP 2.0.1 (Balloux 2001). We ran ten simulation-replicates for 200 generations (equivalent to 200 years for *N. wollastoni* and possibly 100 or 200 years for *M. gracilis*, which breed at most once and maybe twice a year respectively) for six scenarios. In each case, the first generation was a panmictic population of n_0 individuals scored at ten loci each with ten alleles, equivalent to the average number of alleles in the real data set. This population was split in two ($n_1 = n_0/2$ each) with a given migration rate (m) between them for 200 generations. The six scenarios were: $n_0 = 4000$; $m = 0$ (designated as $S^{4K,0m}$); $n_0 = 4000$; $m = 0.001$ ($S^{4K,0.001m}$); $n_0 = 4000$; $m = 0.1$ ($S^{4K,0.1m}$); $n_0 = 20{,}000$, $m = 0$ ($S^{20K,0m}$); $n_0 = 20{,}000$, $m = 0.1$ ($S^{20K,0.1m}$); and $n_0 = 200{,}000$, $m = 0$ ($S^{200K,0m}$). Whenever F_{ST} of the 200th generation was > 0, a subset of genotypes matching the number of individuals actually sampled (400 for *N. wollastoni*, so 200 from each simulated population, and 700 for *M. gracilis*, 350 from each simulated population) were analysed using STRUCTURE as for the real data. We ran three chains of the model with admixture of ancestry and correlation of allele frequencies, for 100,000 burns-in, followed by 100,000 iterations. If, in each case when $m = 0$, we obtain $K = 2$ and the STRUCTURE plots reveal two distinct genetic groups representing the two populations, it would indicate that our actual markers provide enough power to observe known genetic structure and 200 generations is long enough to observe the effects of genetic drift despite large effective population sizes. If, for a given simulation scenario, there is a marked difference in genetic structure between results obtained with three loci (e.g. if $K = 1$) compared to those with ten loci (e.g. if $K = 2$), then three markers are inadequate. Detecting no genetic structure in scenario $S^{200K,0m}$ would mean that either 200 generations is not long enough or 10 markers are insufficient. To distinguish between these two possibilities, we ran scenario $S^{200K,0m}$ for 200 generations with 60 markers ($S^{200K,0m,\ 60\ markers}$) and compared results to those obtained with three and 10 markers. Our actual reduced dataset for *N. wollastoni*, which does not have any loci

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with null alleles (see Results and Accessory Publication), is very similar to the simulated dataset with three markers.

Results

Simulations to test ability of our markers to detect genetic structure

Both EASYPOP simulations involving small-sized populations split into two with no or little migration respectively between them ($S^{4K,0m}$ and $S^{4K,0.001m}$) produced two genetic clusters in STRUCTURE that were invariably diagnosed with either three or ten markers. The membership of each genetic cluster matched the 'geographic' location of simulated genotypes. Increasing the migration rate ($S^{4K,0.1m}$) erased genetic structure, i.e. only one population was detected. Similarly, the scenario involving high m and large n_0 ($S^{20K,0.1m}$) resulted in no detectable genetic differentiation with either three or ten loci (ie. F_{ST} was zero). For each of the remaining scenarios ($S^{20K,0m}$ and $S^{200K,0m}$), although F_{ST} was greater than zero, only one genetic cluster was evident after STRUCTURE analysis including either three or ten markers. This was also the case for $S^{200K,0m,60markers}$, indicating that even 60 markers could not detect genetic structure. In these scenarios, 200 generations was not long enough to create detectable genetic differentiation of populations of the given sizes.

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Table 1: Details of markers in *Necterosoma wollastoni* and *Micronecta gracilis*. H_E is expected heterozygosity, H_O is observed heterozygosity. If H_E significantly exceeds H_O, the locus is not in Hardy-Weinberg equilibrium. Reasons for data missing at a given locus: (nvp) no visible PCR product; and (sc) PCR product visible but impossible to score.

Locus name	No. alleles	H_E/H_O	*p*	data missing (reason)
Necterosoma wollastoni				
NW20	5	0.57/0.54	0.26	0%
NW30	10	0.52/0.23	0	1% (nvp)
NW60	10	0.26/0.26	0.41	0%
NW64	15	0.82/0.70	0	11% (sc)
Micronecta gracilis				
MG27	13	0.66/0.10	0	29% (nvp)
MG37	13	0.44/0.24	0	9% (nvp)
MG40	28	0.86/0.27	0	41% (nvp)
MG44	3	0.30/0.27	0.001	3% (nvp)
MG51	19	0.82/0.18	0	31% (nvp)
MG53	24	0.83/0.59	0	9% (nvp)

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Table 2 Mitochondrial DNA sequence haplotype frequencies of *N. wollastoni* in each landscape.

	Landscape										n
Haplotype	**Ag[1]**	**Fo[2]**	**Fo[3]**	**Ag[4]**	**Fo[5]**	**Ag[6]**	**Fo[7]**	**Fo[8]**	**Ag[9]**	**Ag[10]**	**individuals**
1	0.67	0.56	0.57	0.63	0.54	0.50	0.55	0.62	0.51	0.56	141
2	0.33	0.06	0.06	0.13	0.06	0.08	0.03		0.04	0.11	17
3		0.06	0.04		0.13	0.08	0.07	0.08	0.06		15
4		0.06	0.06		0.06		0.03		0.02	0.11	10
5			0.02	0.13			0.03		0.02		5
6			0.02		0.02				0.04		4
7			0.02				0.03		0.02		4
8			0.02		0.02	0.04					4
9			0.02				0.03		0.04		3
10					0.02		0.03	0.08			3
11					0.02	0.04		0.08			3
12		0.19	0.02			0.04					3
13		0.06			0.02						3
14						0.04	0.03				2
15				0.13			0.03				2
16			0.02		0.02						2
17					0.02			0.08			2
18					0.02				0.04		2
19			0.02						0.02		2
20			0.02						0.02		2
21			0.02								2
22			0.02								2
23			0.02								2
24			0.02								1
25			0.02								1
26			0.02								1
27					0.02						1
28					0.02						1
29						0.04					1
30						0.04					1
31						0.04					1
32						0.04					1
33							0.03				1
34							0.07				1
35								0.08			1
36									0.02		1
37									0.04		1
38									0.02		1
39									0.04		1
40									0.02		1
41									0.02		1
42									0.02		1
43									0.02		1
44										0.11	1
45										0.11	1

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n individuals	3	16	54	8	48	24	29	13	53	9	257
n haplotypes	2	6	19	4	14	11	12	6	19	5	

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Table 3 Mitochondrial DNA sequence haplotype frequencies of *M. gracilis* in each landscape.

	Landscape										n
Haplotype	**Ag[1]**	**Fo[2]**	**Fo[3]**	**Ag[4]**	**Fo[5]**	**Ag[6]**	**Fo[7]**	**Fo[8]**	**Ag[9]**	**Ag[10]**	individuals
1	0.22	0.58	0.55	0.51	0.67	0.74	0.52	0.77	0.67	0.60	235
2	0.03	0.12	0.11	0.07	0.03		0.03	0.02			17
3	0.06		0.02	0.05	0.03	0.11			0.10	0.05	15
4	0.03		0.14				0.03	0.02		0.02	10
5				0.07	0.06	0.03				0.02	7
6			0.05				0.03	0.02		0.02	5
7		0.04							0.02	0.02	3
8			0.02	0.02	0.03						3
9				0.02		0.03		0.02			3
10	0.03			0.02							2
11	0.03									0.02	2
12		0.04		0.02							2
13			0.02	0.02							2
14			0.02		0.03						2
15				0.02				0.02			2
16					0.03	0.03					2
17						0.03			0.02		2
18		0.04								0.02	2
19								0.02	0.02		2
20	0.03										2
21	0.03										1
22	0.03										1
23	0.03										1
24	0.03										1
25	0.03										1
26	0.03										1
27	0.03										1
28	0.03										1
29	0.03										1
30	0.03										1
31	0.03										1
32	0.03										1
33	0.03										1
34	0.03										1
35	0.03										1
36	0.03										1
37	0.03										1
38	0.03										1
39	0.03										1
40	0.03										1
41	0.03										1
42		0.04									1
43		0.04									1
44		0.04									1
45		0.04									1
46		0.04									1
47			0.02								1
48			0.02								1
49			0.02								1
50				0.02							1
51				0.02							1

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52				0.05							1
53				0.02							1
54				0.02							1
55				0.02							1
56					0.03						1
57					0.03						1
58					0.03						1
59					0.03						1
60					0.03						1
61						0.03					1
62						0.03					1
63							0.03				1
64							0.03				1
65							0.03				1
66							0.03				1
67							0.03				1
68							0.03				1
69							0.03				1
70							0.03				1
71							0.03				1
72							0.03				1
73							0.03				1
74							0.03				1
75								0.02			1
76								0.02			1
77								0.02			1
78								0.02			1
79								0.02			1
80								0.02			1
81									0.02		1
82									0.02		1
83									0.02		1
84									0.02		1
85									0.02		1
86									0.02		1
87									0.02		1
88									0.02		1
89									0.02		1
90										0.02	1
91										0.02	1
92										0.02	1
93										0.02	1
94										0.02	1
95										0.02	1
96										0.02	1
97										0.02	1
98										0.02	1
n individuals	36	26	44	43	36	38	31	51	51	42	398
n haplotypes	28	10	11	16	12	8	16	13	14	17	