

Supplementary Information 1. Ipyrad parameter settings for datasets used in downstream analyses.

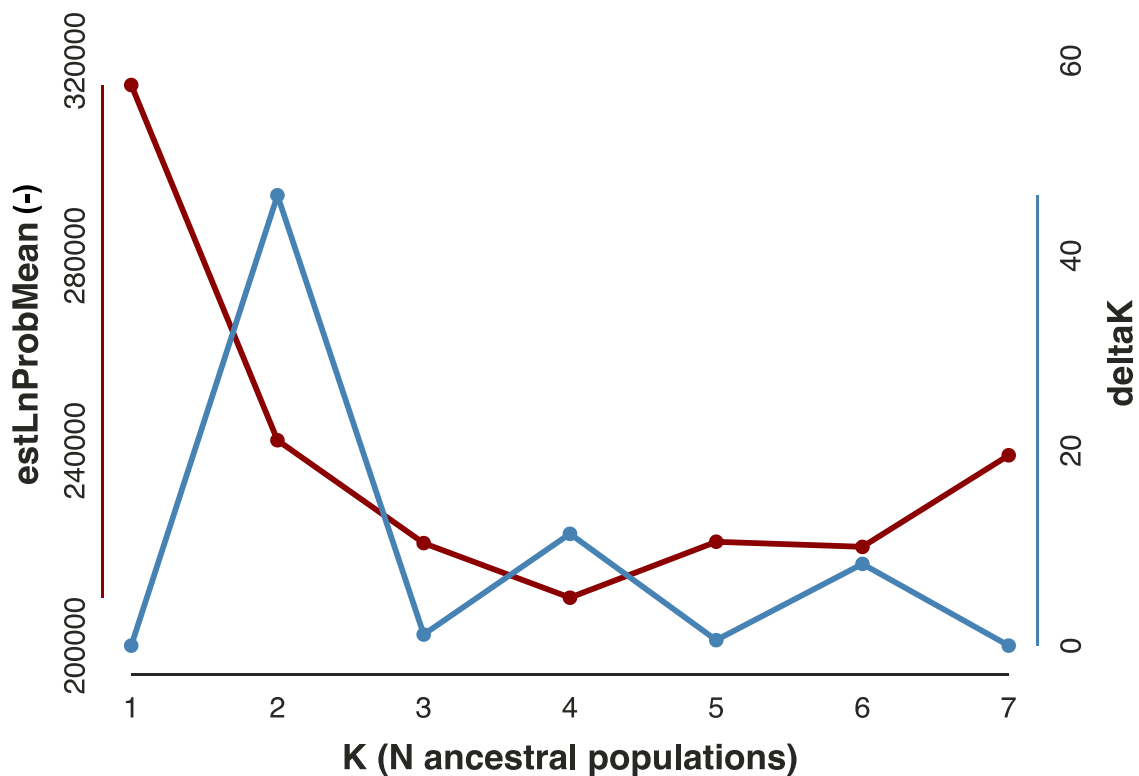
Full Sample Set		Distant Ingroups Subset		Close Ingroups Subset		Target Species Subset	
Parameter	Value	Parameter	Value	Parameter	Value	Parameter	Value
Restriction_overhang	"TGCAG", ""	Restriction_overhang	"TGCAG", ""	Restriction_overhang	"TGCAG", ""	Restriction_overhang	"TGCAG", ""
Max_low_qual_bases	5	Max_low_qual_bases	5	Max_low_qual_bases	5	Max_low_qual_bases	5
Phred_Qscore_offset	33	Phred_Qscore_offset	33	Phred_Qscore_offset	33	Phred_Qscore_offset	33
mindepth_statistical	6	mindepth_statistical	6	mindepth_statistical	6	mindepth_statistical	6
mindepth_majrule	6	mindepth_majrule	6	mindepth_majrule	6	mindepth_majrule	6
maxdepth	10,000	maxdepth	10,000	maxdepth	10,000	maxdepth	10,000
clust_threshold	0.9	clust_threshold	0.9	clust_threshold	0.9	clust_threshold	0.9
max_barcode_mismatch	0	max_barcode_mismatch	0	max_barcode_mismatch	0	max_barcode_mismatch	0
filter_adapters	2	filter_adapters	2	filter_adapters	2	filter_adapters	2
filter_min_trim_len	35	filter_min_trim_len	35	filter_min_trim_len	35	filter_min_trim_len	35
max_alleles_consens	2	max_alleles_consens	2	max_alleles_consens	2	max_alleles_consens	2
max_Ns_consens	0.05	max_Ns_consens	0.05	max_Ns_consens	0.05	max_Ns_consens	0.05
max_Hs_consens	0.05	max_Hs_consens	0.05	max_Hs_consens	0.05	max_Hs_consens	0.05
min_samples_locus	72	min_samples_locus	64	min_samples_locus	47	min_samples_locus	32
max_SNPs_locus	0.2	max_SNPs_locus	0.2	max_SNPs_locus	0.2	max_SNPs_locus	0.2
max_Indels_locus	8	max_Indels_locus	8	max_Indels_locus	8	max_Indels_locus	8
max_shared_Hs_locus	0.5	max_shared_Hs_locus	0.5	max_shared_Hs_locus	0.5	max_shared_Hs_locus	0.5
trim_reads	0,0,0,0	trim_reads	0,0,0,0	trim_reads	0,0,0,0	trim_reads	0,0,0,0
trim_loci	0,0,0,0	trim_loci	0,0,0,0	trim_loci	0,0,0,0	trim_loci	0,0,0,0
Samples from the following species included:	All Samples	Samples from the following species included:	<i>Z. tuberculata</i> <i>Z. littoralis</i> <i>Z. granulata</i> <i>Z. aff. tuberculata</i> <i>Z. parrisiae</i> <i>Z. buxijugum</i> <i>Z. formosa</i>	Samples from the following species included:	<i>Z. granulata</i> <i>Z. aff. tuberculata</i> <i>Z. parrisiae</i> <i>Z. buxijugum</i> <i>Z. formosa</i>	Samples from the following species included:	<i>Z. parrisiae</i> <i>Z. buxijugum</i> <i>Z. formosa</i>

Supplementary Information 2. Information on K selection for the output of STRUCTURE analysis of the close ingroups dataset using custom approach. **(a)** Summary table of mean values for each K-value across 99 Evanno tables. **(b)** Plot of mean estLnProbMean (Ln P(K)) and deltaK (from (a)) for each value of K. **(c)** Statistics for Evanno tables produced under different max_var_multiple (MVM) values. The selected MVM value (73) is highlighted in yellow. **(d)** Plot of highest estLnProbMean (Ln P(K)) and deltaK for each tested MVM. Note: The MVM value of 1 was removed due to an insufficient number of runs reaching convergence (see table (c)).

(a)

K-value	Number of runs retained	lnPK	lnPPK	deltaK	estLnProbMean	estLnProbStdev
1	10	0	0	0	-314985	1304.161
2	10	72871.99	51785.73	46.185	-242113	1121.275
3	10	21086.26	9892.326	1.137	-221027	8697.375
4	9.01	11193.93	22660.96	11.468	-209833	8794.023
5	8.02	-11467	17288.58	0.56	-221300	36132.54
6	7.03	1045.457	39002.76	8.377	-220255	28927.74
7	8.182	-18789.2	0	0	-239044	67573.54

(b)



(c)

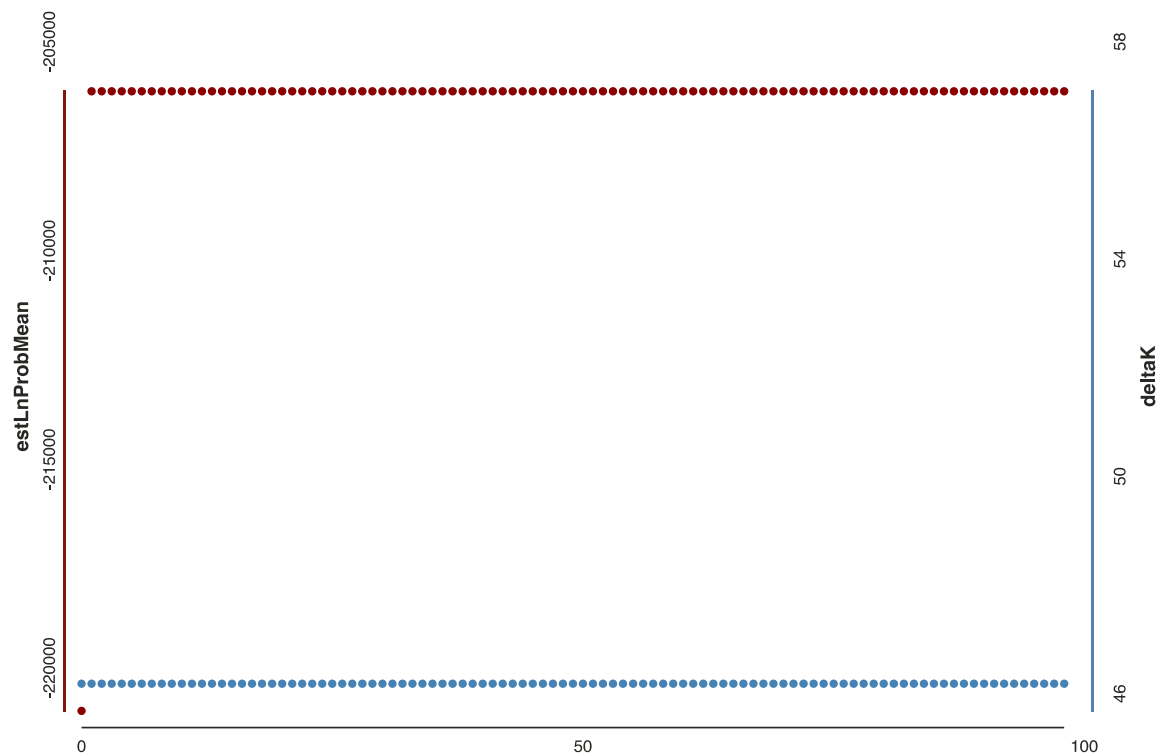
Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
0	10	0	-221027	3	46.185	2
1	1	7	-207118	5	0	1
2	8.714	0	-206187	5	46.185	2
3	8.714	0	-206187	5	46.185	2
4	8.714	0	-206187	5	46.185	2
5	8.714	0	-206187	5	46.185	2
6	8.714	0	-206187	5	46.185	2
7	8.714	0	-206187	5	46.185	2
8	8.714	0	-206187	5	46.185	2
9	8.714	0	-206187	5	46.185	2
10	8.714	0	-206187	5	46.185	2
11	8.714	0	-206187	5	46.185	2
12	8.714	0	-206187	5	46.185	2
13	8.714	0	-206187	5	46.185	2
14	8.714	0	-206187	5	46.185	2
15	8.714	0	-206187	5	46.185	2
16	8.714	0	-206187	5	46.185	2
17	8.714	0	-206187	5	46.185	2
18	8.714	0	-206187	5	46.185	2
19	8.714	0	-206187	5	46.185	2
20	8.714	0	-206187	5	46.185	2
21	8.714	0	-206187	5	46.185	2
22	8.714	0	-206187	5	46.185	2
23	8.714	0	-206187	5	46.185	2
24	8.714	0	-206187	5	46.185	2
25	8.714	0	-206187	5	46.185	2
26	8.714	0	-206187	5	46.185	2
27	8.714	0	-206187	5	46.185	2
28	8.714	0	-206187	5	46.185	2
29	8.714	0	-206187	5	46.185	2
30	8.714	0	-206187	5	46.185	2
31	8.714	0	-206187	5	46.185	2
32	8.714	0	-206187	5	46.185	2
33	8.714	0	-206187	5	46.185	2
34	8.714	0	-206187	5	46.185	2
35	8.714	0	-206187	5	46.185	2
36	8.857	0	-206187	5	46.185	2
37	8.857	0	-206187	5	46.185	2

Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
38	8.857	0	-206187	5	46.185	2
39	8.857	0	-206187	5	46.185	2
40	8.857	0	-206187	5	46.185	2
41	8.857	0	-206187	5	46.185	2
42	8.857	0	-206187	5	46.185	2
43	8.857	0	-206187	5	46.185	2
44	8.857	0	-206187	5	46.185	2
45	8.857	0	-206187	5	46.185	2
46	8.857	0	-206187	5	46.185	2
47	8.857	0	-206187	5	46.185	2
48	8.857	0	-206187	5	46.185	2
49	8.857	0	-206187	5	46.185	2
50	9	0	-206187	5	46.185	2
51	9	0	-206187	5	46.185	2
52	9	0	-206187	5	46.185	2
53	9	0	-206187	5	46.185	2
54	9	0	-206187	5	46.185	2
55	9	0	-206187	5	46.185	2
56	9	0	-206187	5	46.185	2
57	9	0	-206187	5	46.185	2
58	9	0	-206187	5	46.185	2
59	9	0	-206187	5	46.185	2
60	9	0	-206187	5	46.185	2
61	9	0	-206187	5	46.185	2
62	9	0	-206187	5	46.185	2
63	9	0	-206187	5	46.185	2
64	9	0	-206187	5	46.185	2
65	9	0	-206187	5	46.185	2
66	9	0	-206187	5	46.185	2
67	9	0	-206187	5	46.185	2
68	9	0	-206187	5	46.185	2
69	9	0	-206187	5	46.185	2
70	9	0	-206187	5	46.185	2
71	9	0	-206187	5	46.185	2
72	9	0	-206187	5	46.185	2
73	9	0	-206187	5	46.185	2
74	9	0	-206187	5	46.185	2
75	9	0	-206187	5	46.185	2

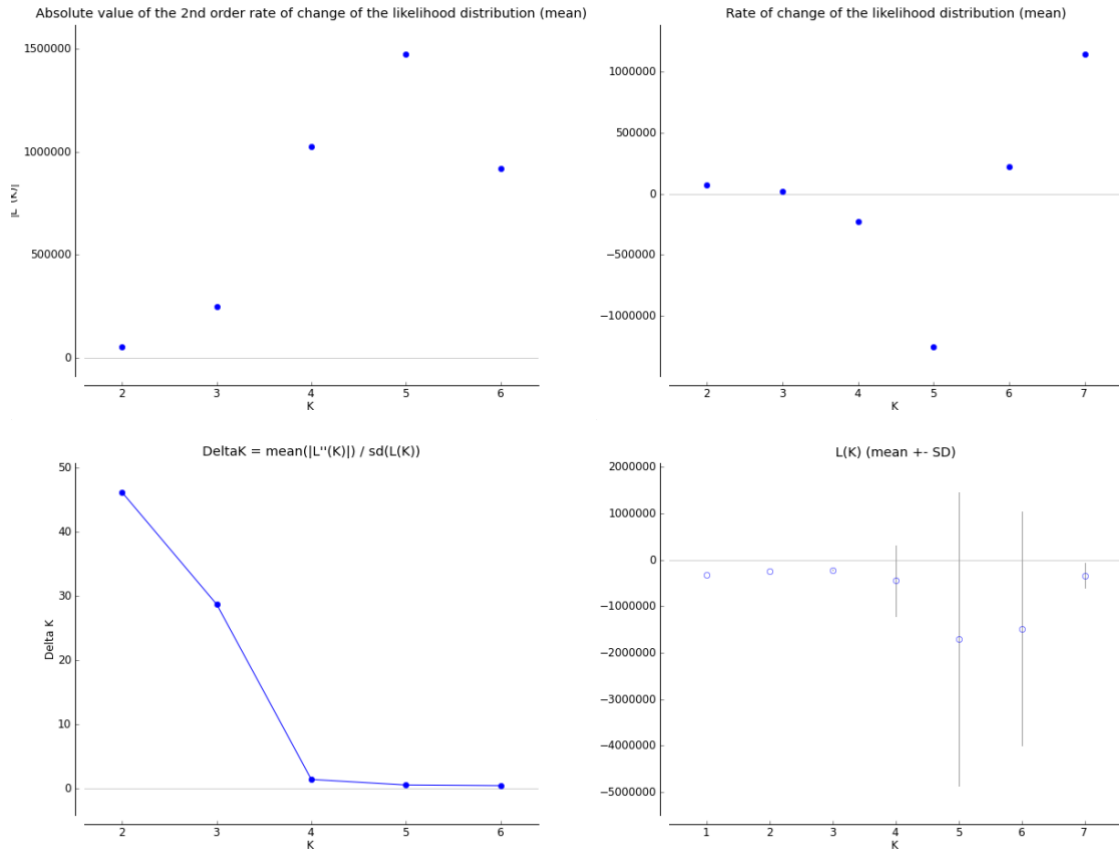
Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
76	9	0	-206187	5	46.185	2
77	9	0	-206187	5	46.185	2
78	9	0	-206187	5	46.185	2
79	9	0	-206187	5	46.185	2
80	9	0	-206187	5	46.185	2
81	9	0	-206187	5	46.185	2
82	9	0	-206187	5	46.185	2
83	9	0	-206187	5	46.185	2
84	9	0	-206187	5	46.185	2
85	9	0	-206187	5	46.185	2
86	9	0	-206187	5	46.185	2
87	9	0	-206187	5	46.185	2

Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
88	9	0	-206187	5	46.185	2
89	9	0	-206187	5	46.185	2
90	9	0	-206187	5	46.185	2
91	9	0	-206187	5	46.185	2
92	9	0	-206187	5	46.185	2
93	9	0	-206187	5	46.185	2
94	9	0	-206187	5	46.185	2
95	9	0	-206187	5	46.185	2
96	9	0	-206187	5	46.185	2
97	9	0	-206187	5	46.185	2
98	9	0	-206187	5	46.185	2
99	9	0	-206187	5	46.185	2

(d)



Supplementary Information 3. Structure Harvester output for STRUCTURE analysis of the ‘close ingroups’ dataset. The yellow row on the Evanno table indicates highest deltaK, and therefore the suggested K value.



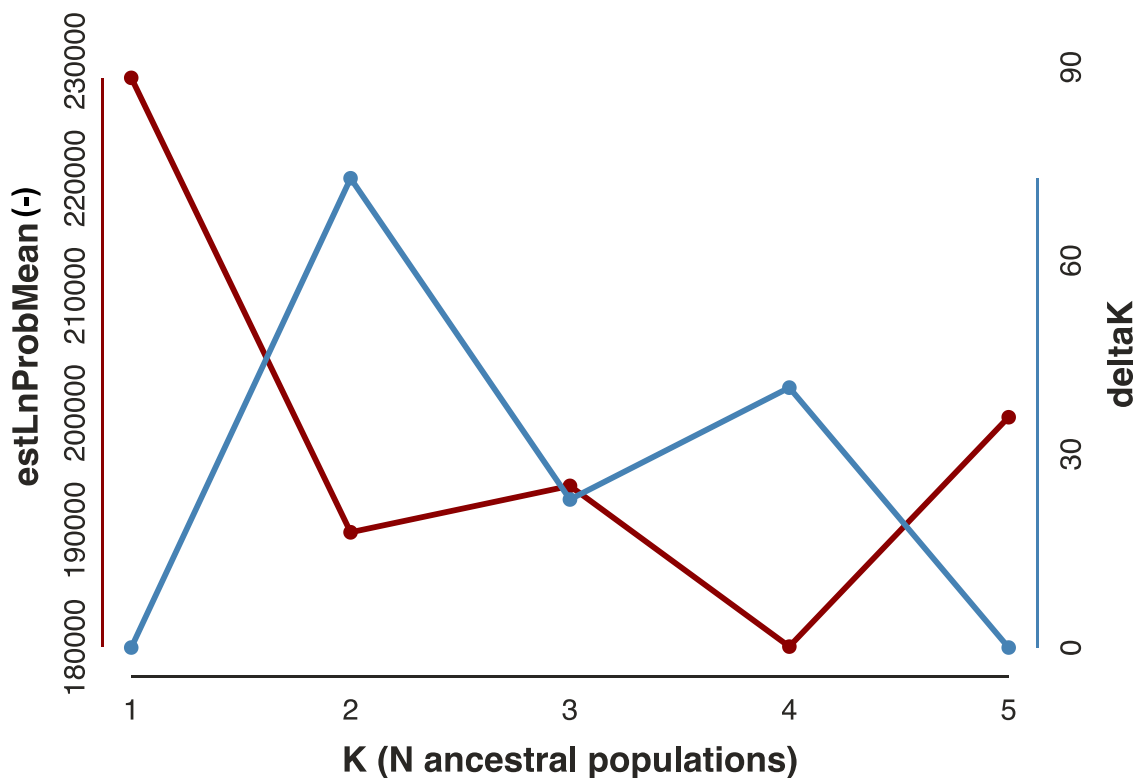
K	Rep s	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	10	-314985.16	1304.1614	—	—	—
2	10	-242113.17	1121.2749	72871.99	51785.73	46.184687
3	10	-221026.91	8697.375	21086.26	249449.87	28.681052
4	10	-449390.52	765278.2173	-228363.61	1024609.17	1.338871
5	10	-1702363.3	3155749.763	-1252972.78	1475152.6	0.467449
6	10	-1480183.48	2516118.102	222179.82	920083.89	0.365676
7	10	-337919.77	265026.3011	1142263.71	—	—

Supplementary Information 4. Information on K selection for the output of STRUCTURE analysis of the target species dataset using custom approach. **(a)** Summary table of mean values for each K-value across 99 Evanno tables. **(b)** Plot of mean estLnProbMean (Ln P(K)) and deltaK (from (a)) for each value of K. **(c)** Statistics for Evanno tables produced under different max_var_multiple (MVM) values. The selected MVM value (73) is highlighted in yellow. **(d)** Plot of highest estLnProbMean (Ln P(K)) and deltaK for each tested MVM. Note: The MVM value of 1 was removed due to an insufficient number of runs reaching convergence (see table (c)), and deltaK axis is skewed above 85 to show deltaK for the MVM of '0' (2939.204).

(a)

K-value	Number of runs retained	lnPK	lnPPK	deltaK	estLnProbMean	estLnProbStdev
1	10	0	0	0	-228563	695.887
2	10	38736.66	42692.44	72.754	-189827	586.804
3	8.02	-3955.78	44504.33	22.961	-193782	37251.74
4	7.03	13696.73	33600.96	40.299	-180086	5644.035
5	6.535	-19549.4	0	0	-199635	56421.47

(b)



(c)

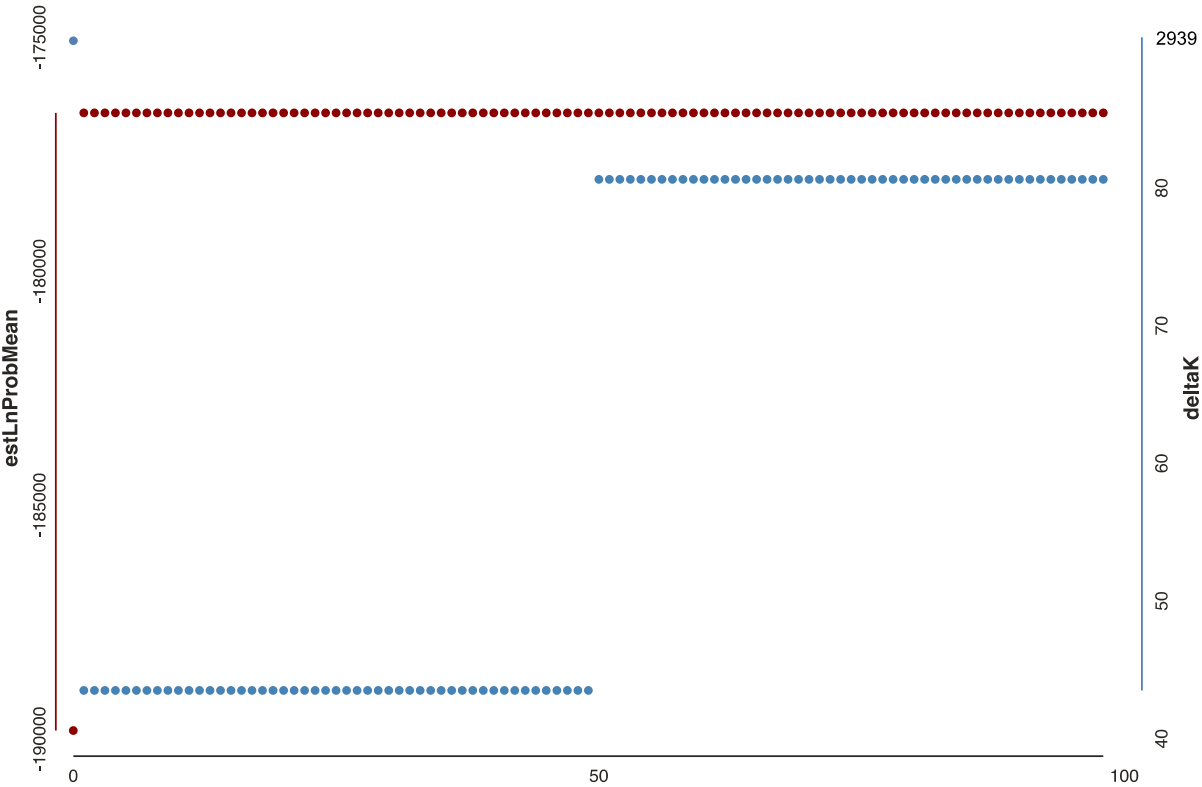
Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
0	10	0	-189827	2	2939.204	2
1	1	5	-176366	3	0	1
2	8.2	0	-176619	3	43.505	2
3	8.2	0	-176619	3	43.505	2
4	8.2	0	-176619	3	43.505	2
5	8.2	0	-176619	3	43.505	2
6	8.2	0	-176619	3	43.505	2
7	8.2	0	-176619	3	43.505	2
8	8.2	0	-176619	3	43.505	2
9	8.2	0	-176619	3	43.505	2
10	8.2	0	-176619	3	43.505	2
11	8.2	0	-176619	3	43.505	2
12	8.2	0	-176619	3	43.505	2
13	8.2	0	-176619	3	43.505	2
14	8.2	0	-176619	3	43.505	2
15	8.2	0	-176619	3	43.505	2
16	8.2	0	-176619	3	43.505	2
17	8.2	0	-176619	3	43.505	2
18	8.2	0	-176619	3	43.505	2
19	8.2	0	-176619	3	43.505	2
20	8.2	0	-176619	3	43.505	2
21	8.2	0	-176619	3	43.505	2
22	8.2	0	-176619	3	43.505	2
23	8.2	0	-176619	3	43.505	2
24	8.2	0	-176619	3	43.505	2
25	8.2	0	-176619	3	43.505	2
26	8.2	0	-176619	3	43.505	2
27	8.2	0	-176619	3	43.505	2
28	8.2	0	-176619	3	43.505	2
29	8.2	0	-176619	3	43.505	2
30	8.2	0	-176619	3	43.505	2
31	8.2	0	-176619	3	43.505	2
32	8.2	0	-176619	3	43.505	2
33	8.2	0	-176619	3	43.505	2
34	8.2	0	-176619	3	43.505	2
35	8.2	0	-176619	3	43.505	2
36	8.2	0	-176619	3	43.505	2
37	8.2	0	-176619	3	43.505	2

Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
38	8.2	0	-176619	3	43.505	2
39	8.2	0	-176619	3	43.505	2
40	8.2	0	-176619	3	43.505	2
41	8.2	0	-176619	3	43.505	2
42	8.2	0	-176619	3	43.505	2
43	8.2	0	-176619	3	43.505	2
44	8.2	0	-176619	3	43.505	2
45	8.2	0	-176619	3	43.505	2
46	8.2	0	-176619	3	43.505	2
47	8.2	0	-176619	3	43.505	2
48	8.2	0	-176619	3	43.505	2
49	8.2	0	-176619	3	43.505	2
50	8.2	0	-176619	3	43.505	2
51	8.4	0	-176619	3	80.621	4
52	8.4	0	-176619	3	80.621	4
53	8.4	0	-176619	3	80.621	4
54	8.4	0	-176619	3	80.621	4
55	8.4	0	-176619	3	80.621	4
56	8.4	0	-176619	3	80.621	4
57	8.4	0	-176619	3	80.621	4
58	8.4	0	-176619	3	80.621	4
59	8.4	0	-176619	3	80.621	4
60	8.4	0	-176619	3	80.621	4
61	8.4	0	-176619	3	80.621	4
62	8.4	0	-176619	3	80.621	4
63	8.4	0	-176619	3	80.621	4
64	8.4	0	-176619	3	80.621	4
65	8.4	0	-176619	3	80.621	4
66	8.4	0	-176619	3	80.621	4
67	8.4	0	-176619	3	80.621	4
68	8.4	0	-176619	3	80.621	4
69	8.4	0	-176619	3	80.621	4
70	8.4	0	-176619	3	80.621	4
71	8.4	0	-176619	3	80.621	4
72	8.4	0	-176619	3	80.621	4
73	8.4	0	-176619	3	80.621	4
74	8.4	0	-176619	3	80.621	4
75	8.4	0	-176619	3	80.621	4

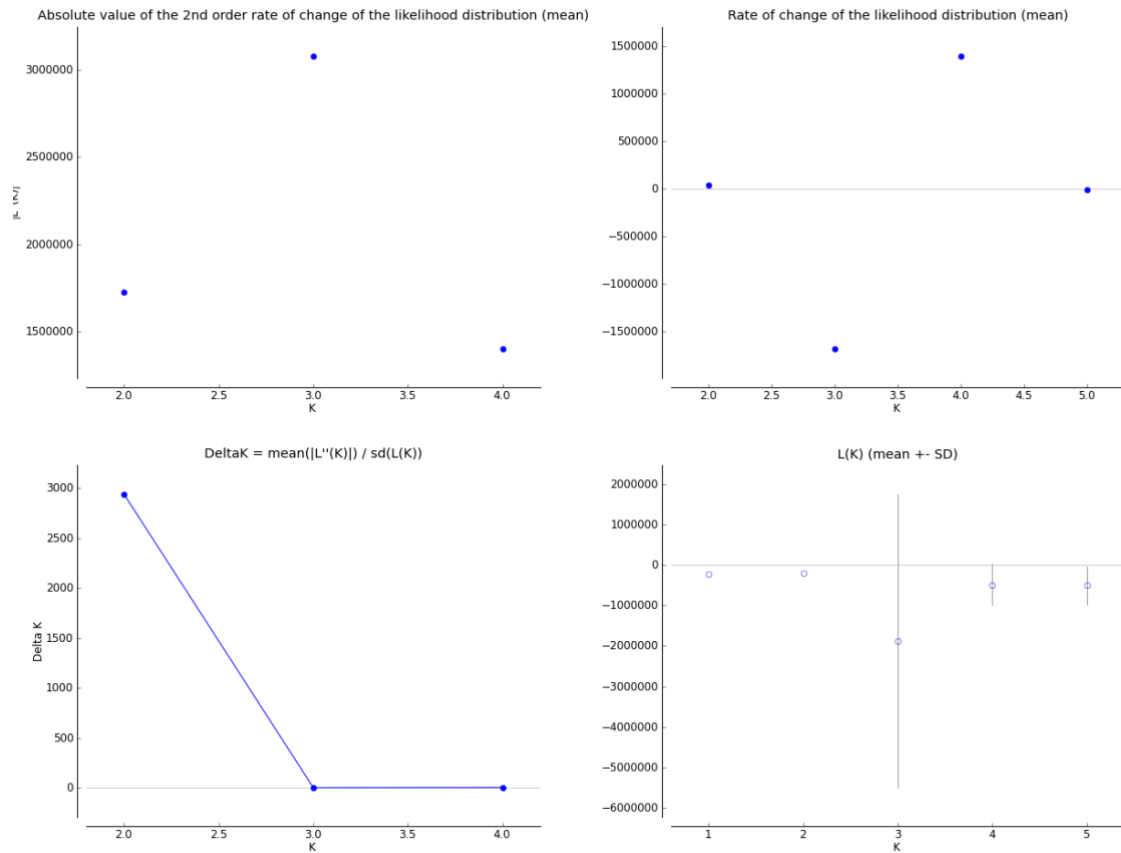
Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
76	8.4	0	-176619	3	80.621	4
77	8.4	0	-176619	3	80.621	4
78	8.4	0	-176619	3	80.621	4
79	8.4	0	-176619	3	80.621	4
80	8.4	0	-176619	3	80.621	4
81	8.4	0	-176619	3	80.621	4
82	8.4	0	-176619	3	80.621	4
83	8.4	0	-176619	3	80.621	4
84	8.4	0	-176619	3	80.621	4
85	8.4	0	-176619	3	80.621	4
86	8.4	0	-176619	3	80.621	4
87	8.4	0	-176619	3	80.621	4

Max var multiple	Mean # runs retained	Failed # of runs	Highest mean Ln P (K)	K value for Ln P (K)	Highest mean deltaK	K value for deltaK
88	8.4	0	-176619	3	80.621	4
89	8.4	0	-176619	3	80.621	4
90	8.4	0	-176619	3	80.621	4
91	8.4	0	-176619	3	80.621	4
92	8.4	0	-176619	3	80.621	4
93	8.4	0	-176619	3	80.621	4
94	8.4	0	-176619	3	80.621	4
95	8.4	0	-176619	3	80.621	4
96	8.4	0	-176619	3	80.621	4
97	8.4	0	-176619	3	80.621	4
98	8.4	0	-176619	3	80.621	4
99	8.4	0	-176619	3	80.621	4

(d)



Supplementary Information 5. Structure Harvester output for STRUCTURE analysis of the ‘target species’ dataset. The yellow row on the Evanno table indicates highest deltaK, and therefore the suggested K value.



K	Rep s	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	10	-228563.37	695.8866	—	—	—
2	10	-189826.71	586.8036	38736.66	1724735.4 4	2939.20410 5
3	10	-1875825.49	3630597.337	-1685998.78	3076763.3 3	0.847454
4	10	-485060.94	511453.3891	1390764.55	1401991.0 4	2.74119
5	10	-496287.43	464238.0196	-11226.49	—	—

Supplementary Data 6. Cross-validation plot for TESS3 analysis of the target species dataset.

