Solving the genetic specimen sample size problem for DNA barcoding with a local search optimization algorithm

Supplementary Information

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Figures

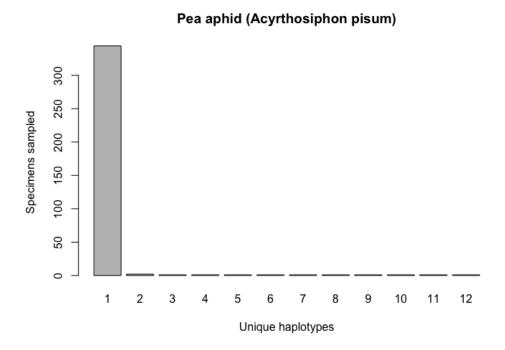


Figure 1: Initial haplotype frequency distribution for N=356 high-quality Pea aphid (*Acyrthosiphon pisum*) COI barcode sequences obtained from BOLD. This species displays a highly-skewed pattern of observed haplotype variation, Haplotype 1 accounts for c.~96.6% (344/356) of all sampled records.

Common mosquito (Culex pipiens) Specimens sampled

Figure 2: Initial haplotype frequency distribution for N=217 high-quality Common mosquito (*Culex pipiens*) COI barcode sequences obtained from BOLD. In this species, Haplotype 1 accounts for c.~84.3%~(183/217) of all sampled records.

Unique haplotypes

Gypsy moth (Lymantria dispar) 8 9 07 1 4 7 10 14 18 22 26 30 34 38 42 46 50 54 58

Figure 3: Initial haplotype frequency distribution for N=365 high-quality Gypsy moth (*Lymantria dispar*) COI barcode sequences obtained from BOLD. In this species, Haplotypes 1-5 account for c.~70.4%~(257/365) of all sampled records.

Unique haplotypes

Scenario II (1 dominant haplotype)

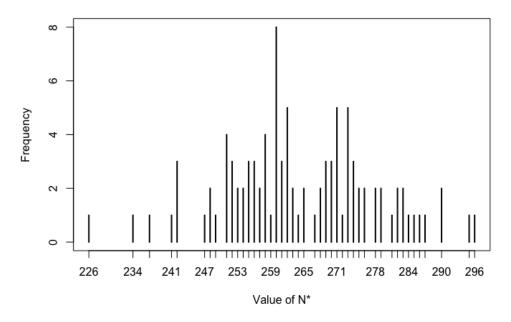


Figure 4: Frequency plot showing all located local optima and the number of times each was found by HACSim for Scenario II (1 dominant haplotype).

Scenario III (2 dominant haplotypes)

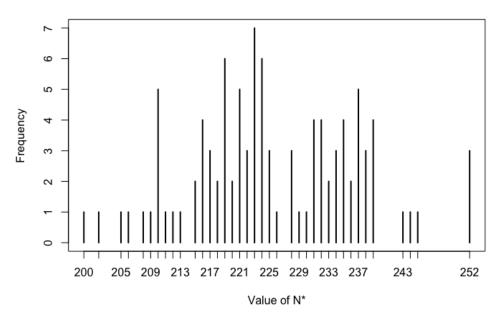


Figure 5: Frequency plot showing all located local optima and the number of times each was found by HACSim for Scenario III (2 dominant haplotypes).

Scenario IV (3 dominant haplotypes)

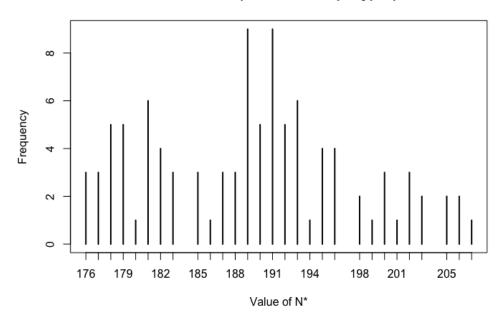


Figure 6: Frequency plot showing all located local optima and the number of times each was found by HACSim for Scenario IV (3 dominant haplotypes).

Pea aphid (Acyrthosiphon pisum)

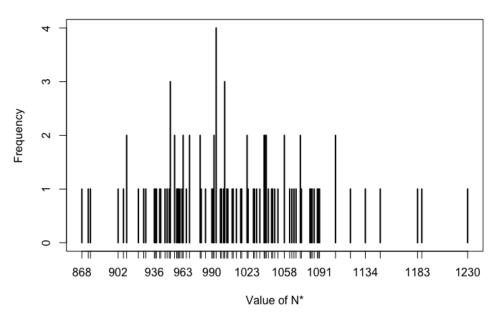


Figure 7: Frequency plot showing all located local optima and the number of times each was found by HACSim for Pea aphid (*Acyrthosiphon pisum*).

Common mosquito (Culex pipiens)

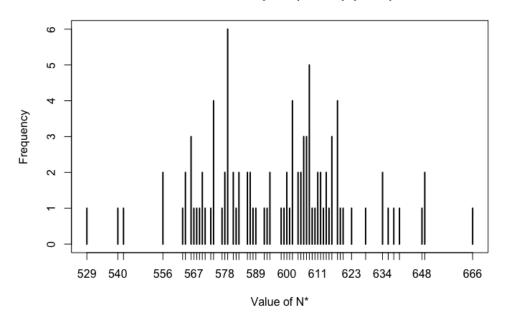


Figure 8: Frequency plot showing all located local optima and the number of times each was found by HACSim for Common mosquito (*Culex pipiens*).

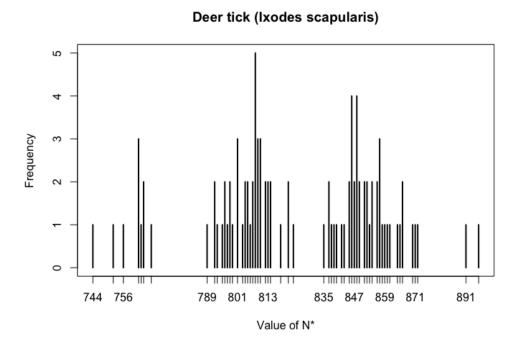


Figure 9: Frequency plot showing all located local optima and the number of times each was found by HACSim for Deer tick (*Ixodes scapularis*).

Gypsy moth (Lymantria dispar)

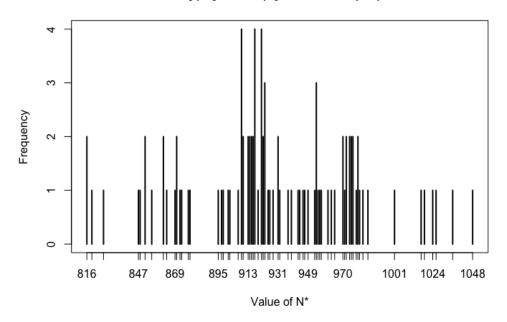


Figure 10: Frequency plot showing all located local optima and the number of times each was found by HACSim for Gypsy moth (*Lymantria dispar*).

Scalloped hammerhead shark (Sphyrna lewini)

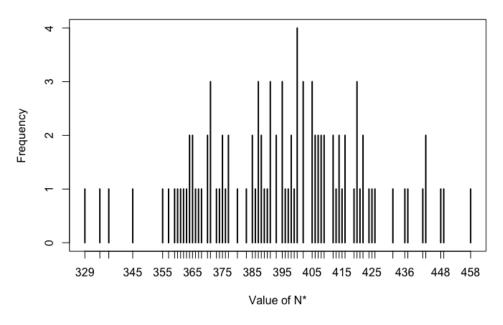


Figure 11: Frequency plot showing all located local optima and the number of times each was found by HACSim for Scalloped hammerhead shark (*Sphyrna lewini*).

Scenario II (1 dominant haplotype) Population size: 10000

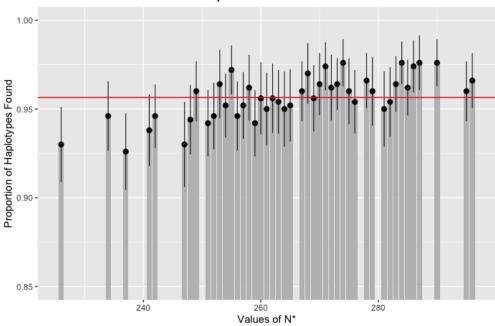


Figure 12: Plot showing all located local optima and the proportion of observed haplotypes captured for Scenario II (1 dominant haplotype) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 based on the number of local optima and holds no additional meaning in the context of this study.

Scenario III (2 dominant haplotypes)

Figure 13: Plot showing all located local optima and the proportion of observed haplotypes captured for Scenario III (2 dominant haplotypes) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 based on the number of local optima and holds no additional meaning in the context of this study.

220

Values of N*

240

250

0.85 -

200

210

Scenario IV (3 dominant haplotypes) Population size: 10000

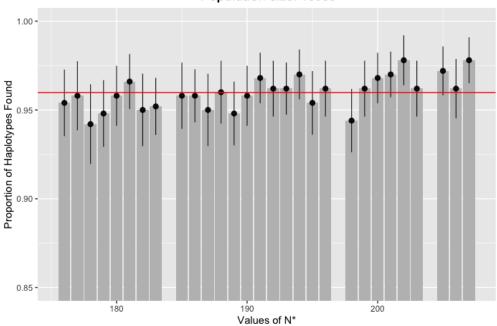


Figure 14: Plot showing all located local optima and the proportion of observed haplotypes captured for Scenario IV (3 dominant haplotypes) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 based on the number of local optima and holds no additional meaning in the context of this study.

Pea aphid (Acyrthosiphon pisum) Population size: 10000 1.00 1.00 0.90 0.85-

Figure 15: Plot showing all located local optima and the proportion of observed haplotypes captured for Pea aphid (*A. pisum*) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 based on the number of local optima and holds no additional meaning in the context of this study.

850

1050 Values of N*

Common mosquito (Culex pipiens) Population size: 10000 1.

Figure 16: Plot showing all located local optima and the proportion of observed haplotypes captured for Common mosquito (*C. pipiens*) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 based on the number of local optima and holds no additional meaning in the context of this study.

Deer tick (Ixodes scapularis)

Figure 17: Plot showing all located local optima and the proportion of observed haplotypes captured for Deer tick (*I. scapularis*) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 based on the number of local optima and holds no additional meaning in the context of this study.

Values of N*

0.85 -

Gypsy moth (Lymantria dispar) Population size: 10000 1.00 0.95 0.85 850 900 Values of N*

Figure 18: Plot showing all located local optima and the proportion of observed haplotypes captured for Gypsy moth (*L. dispar*) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 and thus holds no additional meaning in the context of this study.

Scalloped hammerhead shark (Sphyrna lewini) Population size: 10000

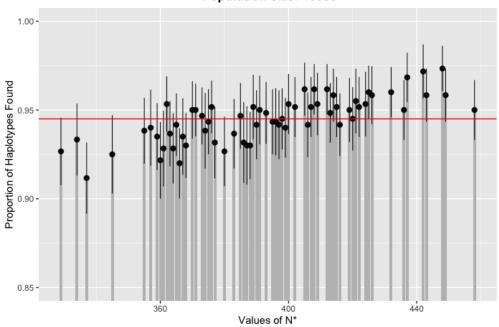


Figure 19: Plot showing all located local optima and the proportion of observed haplotypes captured for Scalloped hammerhead shark (S. lewini) with 95% confidence intervals for a population size of 10000. The mean proportion across 50 replications is depicted as a black dot. The mean proportion across all located local optima is displayed as a solid red horizontal line. The thickness of displayed grey error bars is a construct of ggplot2 and thus holds no additional meaning in the context of this study.

Tables

p = 0.80

Table 1: Mean proportion of observed unique haplotypes found over 50 replications of the simulation study for hypothetical species. In all cases, HACSim was run 100 times. Estimates correspond to the solid red horizontal lines depicted in confidence interval plots found within and **Supplemental Information** accompanying the present article.

Scenario	Pop. size	R Mean	R Range	R Std. dev.
II	1000	0.878	0.840-0.932	0.03148935
II	10000	0.8648	0.812 - 0.910	0.02980922
II	100000	0.8424	0.798 - 0.900	0.03256475
II	10000000	0.8396	0.794 - 0.894	0.0341304
III	1000	0.8493333	0.764 - 0.886	0.03029951
III	10000	0.851	0.770 - 0.892	0.03328663
III	100000	0.8441667	0.768 - 0.864	0.0269202
III	10000000	0.8365	0.784 - 0.868	0.02419805
IV	1000	0.822	0.822 - 0.822	N/A
IV	10000	0.858	$0.858 \ 0.858$	N/A
IV	100000	0.814	0.814 - 0.814	N/A
IV	10000000	0.858	0.858 - 0.858	N/A

all cases, HACSim was run 100 times. Estimates correspond to the solid red horizontal lines depicted in confidence interval plots found within the Supplemental Information accompanying the present article. Table 2: Mean proportion of observed unique haplotypes found over 50 replications of the simulation study for real species. In

Species	Pop. size	R Mean	R Range	R Std. dev.
Pea aphid (Acyrthosiphon pisum)	1000	0.8965344	0.8366667-0.96	0.03823655
Pea aphid ($Acyrthosiphon\ pisum$)	10000	0.8317196	0.765 - 0.895	0.03675206
Pea aphid $(Acyrthosiphon\ pisum)$	100000	0.8342857	0.7616667 - 0.8933333	0.03595436
Pea aphid $(Acyrthosiphon\ pisum)$	10000000	0.8315873	0.773333-0.8966667	0.03537615
Lake whitefish (Coregonus clupeaformis)	1000	0.8322963	0.7840000 - 0.8906667	0.03074708
Lake whitefish (Coregonus clupeaformis)	10000	0.8249259	0.785333 - 0.8746667	0.02958819
Lake whitefish (Coregonus clupeaformis)	100000	0.8384074	0.797333 - 0.8946667	0.03157306
Lake whitefish (Coregonus clupeaformis)	10000000	0.8373333	0.7773333 - 0.8973333	0.03067412
Common mosquito (Culex pipiens)	1000	0.8189943	0.7816 - 0.8592	0.0270726
Common mosquito (Culex pipiens)	10000	0.8341943	0.7816 - 0.8856	0.03253051
Common mosquito (Culex pipiens)	100000	0.83312	0.7816 - 0.8864	0.02916385
Common mosquito (Culex pipiens)	10000000	0.8349714	0.7928 - 0.8792	0.02954371
Deer tick $(Ixodes\ scapularis)$	1000	0.8416466	0.7857831 - 0.8525301	0.01804434
Deer tick $(Ixodes\ scapularis)$	10000	0.8518875	$0.8084337\hbox{-}0.8612048$	0.0146289
Deer tick $(Ixodes\ scapularis)$	100000	0.8463052	$0.8028916 \hbox{-} 0.8575904$	0.01411039
Deer tick (Ixodes scapularis)	10000000	0.8440964	0.8033735 - 0.8590361	0.01460819
Gypsy moth $(Lymantria\ dispar)$	1000	0.8401799	0.8289655 - 0.8551724	0.007725896
Gypsy moth ($Lymantria\ dispar$)	10000	0.8029085	0.7906897 - 0.8172414	0.007518086
Gypsy moth $(Lymantria\ dispar)$	100000	0.8253523	$0.8075862 \ 0.84$	0.008070782
Gypsy moth $(Lymantria\ dispar)$	10000000	0.8274363	0.8151724 - 0.8444828	0.006767465
Scalloped hammerhead shark (Sphyrna lewini)	1000	0.8033333	0.7683333 - 0.8266667	0.02232962
Scalloped hammerhead shark (Sphyrna lewini)	10000	0.8456667	0.8116667 - 0.8633333	0.02046676
Scalloped hammerhead shark (Sphyrna lewini)	100000	0.8386667	0.8083333 - 0.865	0.02283395
Scalloped hammerhead shark (Sphyrna lewini)	10000000	0.9314815	96.0-06.0	0.01565895

Table 3: Bonferroni-corrected Dunn's post-hoc test results at the 3.33% significance level for comparison of population size *versus* haplotype frequency distribution in hypothetical species. **Note**: KW test statistics, *p*-values and statistical significance for Scenario I could not be calculated.

Scenario	Pop. size pairing	Test statistic	<i>p</i> -value	Significant?
II	1000/10000	1.126447	0.7799	No
II	1000/100000	3.025414	0.0074	Yes
II	1000/10000000	3.239814	0.0036	Yes
II	10000/100000	1.898966	0.1727	No
II	10000/10000000	2.113366	0.1037	No
II	100000/10000000	0.214399	1	No
III	1000/10000	-0.167851	1	No
III	1000/100000	0.634915	1	No
III	1000/10000000	1.517958	0.3871	No
III	10000/100000	0.802766	1	No
III	10000/10000000	1.685809	0.2755	No
III	100000/10000000	0.883043	1	No
IV	1000/10000	-0.866025	1	No
IV	1000/100000	0.577350	1	No
IV	1000/10000000	-0.866025	1	No
IV	10000/100000	1.443375	0.4467	No
IV	10000/10000000	0	1	No
IV	100000/10000000	-1.443375	0.4467	No

Table 4: Bonferroni-corrected Dunn's post-hoc test results at the 3.33% significance level for comparison of population size *versus* haplotype frequency distribution in real species.

Species	Pop. size pairing	Test statistic	<i>p</i> -value	Significant?
A. pisum	1000/10000	7.084884	0	Yes
A. pisum	1000/100000	6.743842	0	Yes
A. pisum	1000/10000000	7.127668	0	Yes
A. pisum	10000/100000	-0.341042	1	No
A. pisum	10000/10000000	0.042783	1	No
A. pisum	100000/10000000	0.383825	1	No
C. clupeaformis	1000/10000	1.250438	0.6334	No
C. clupeaformis	1000/100000	-0.873187	1	No
C. clupeaformis	1000/10000000	-0.897207	1	No
C. clupeaformis	10000/100000	-2.123625	0.1011	No
C. clupeaformis	10000/10000000	-2.147645	0.0952	No
C. clupeaformis	100000/10000000	-0.024019	1	No
C. pipiens	1000/10000	-2.667296	0.0229	Yes
C. pipiens	1000/100000	-2.577403	0.0299	Yes
C. pipiens	1000/10000000	-2.830870	0.0139	Yes
C. pipiens	10000/100000	0.089892	1	No
C. pipiens	10000/10000000	-0.163574	1	No
C. pipiens	100000/10000000	-0.253466	1	No
I. scapularis	1000/10000	-2.895996	0.0113	Yes
I. scapularis	1000/100000	-1.196331	0.6947	No
I. scapularis	1000/10000000	-0.372029	1	No
I. scapularis	10000/100000	1.699665	0.2676	No
I. scapularis	10000/10000000	2.523967	0.0348	No
I. scapularis	100000/10000000	0.824301	1	No
$L.\ dispar$	1000/10000	8.129073	0	Yes
$L.\ dispar$	1000/100000	3.854682	0.0003	Yes
$L.\ dispar$	1000/10000000	3.446020	0.0017	Yes
$L.\ dispar$	10000/100000	-4.274390	0.0001	Yes
$L.\ dispar$	10000/10000000	-4.683053	0	Yes
$L.\ dispar$	100000/10000000	-0.408662	1	No
$S.\ lewini$	1000/10000	-2.008237	0.1339	No
$S.\ lewini$	1000/100000	-1.606590	0.3244	No
S. lewini	1000/10000000	-2.811532	0.0148	Yes
$S, \ lewini$	10000/100000	0.401647	1	No
S. lewini	10000/10000000	-0.803295	1	No
S. lewini	100000/10000000	-1.204942	0.6847	No

Table 5: Characteristics of local optima found by HACSim for hypothetical species.

N^* std. dev. Highest mode(s) Highest mode freq.	18	22	100
Highest mode(s)	143	130	100
N^* std. dev.	10.94328	3.620927	0
optima N^* range I	137-182	100 - 136	100-100
No. optima	20	12	1
Scenario	II	III	IV

Table 6: Characteristics of local optima found by HACSim for real species. For some species, distinct optima were located for different population sizes.

Species No.		N^* range	optima N^* range N^* std. dev.	Highest mode(s)	Highest mode freq.
A. pisum	63	499-708	900.99	647	4
$C.\ clupeaformis$	36	309-418	29.27554	325	12
$C.\ pipiens$	35	301 - 399	32.30498	314	~
$I.\ scapularis$	12	349-447	34.61179	439	18
L. dispar	23	468 - 492	5.241212	486	10
S. lewini	ಬ	171-217	13.83943	171	89

Table 7: Coverage probabilities and 95% Clopper-Pearson confidence intervals for hypothetical species for $\mathbf{p} = 0.80$ across all assessed population sizes. Calculated probabilities are based on the number of observed local optima found using HACSim. * Scenario IV comprised only a single local optimum.

Scenario	Pop. size	Coverage prob.	scenario Pop. size Coverage prob. 95% Exact binomial CI Coverage type	Coverage type
II	1000		(0.8315665, 1)	conservative
II	10000	Π	(0.8315665, 1)	conservative
Π	100000	П	(0.8315665, 1)	conservative
II	10000000	Π	(0.8315665, 1)	conservative
III	1000	\vdash	(0.7353515, 1)	conservative
III	10000	\Box	(0.7353515, 1)	conservative
III	100000	\vdash	(0.7353515, 1)	conservative
III	10000000	\vdash	(0.7353515, 1)	conservative
IV^*	1000	\vdash	(0.025, 1)	conservative
\overline{N}	10000	\vdash	(0.025, 1)	conservative
IV	100000	\vdash	(0.025, 1)	conservative
IV	10000000	1	(0.025, 1)	conservative

Table 8: Coverage probabilities and 95% Clopper-Pearson confidence intervals for real species for $\mathbf{p} = 0.80$ across all assessed population sizes. Calculated probabilities are based on the number of observed local optima found using HACSim.

Species	Pop. size	Coverage prob.	95% Exact binomial CI	Coverage type
A. pisum	1000	1	(0.9431276, 1)	conservative
A. pisum	10000	1	(0.9431276, 1)	conservative
A. pisum	100000	1	(0.9431276, 1)	conservative
A. pisum	10000000	1	(0.9431276, 1)	conservative
$C.\ clupeaformis$	1000	1	(0.9026062, 1)	conservative
$C.\ clupeaformis$	10000	1	(0.9026062, 1)	conservative
$C.\ clupeaformis$	100000	1	(0.9026062, 1)	conservative
$C.\ clupeaformis$	10000000	1	(0.9026062, 1)	conservative
$C.\ pipiens$	1000	1	(0.8999676, 1)	conservative
$C.\ pipiens$	10000	1	(0.8999676, 1)	conservative
$C.\ pipiens$	100000	1	(0.8999676, 1)	conservative
$C.\ pipiens$	10000000	1	(0.8999676, 1)	conservative
$I.\ scapularis$	1000	1	(0.7353515, 1)	conservative
$I.\ scapularis$	10000	1	(0.7353515, 1)	conservative
$I.\ scapularis$	100000	1	(0.7353515, 1)	conservative
$I.\ scapularis$	10000000	1	(0.7353515, 1)	conservative
L. dispar	1000	1	(0.8518149, 1)	conservative
L. dispar	10000	1	(0.8518149, 1)	conservative
L. dispar	100000	1	(0.8518149, 1)	conservative
L. dispar	10000000	1	(0.8518149, 1)	conservative
S. lewini	1000	1	(0.4781762, 1)	conservative
$S. \ lewini$	10000	1	(0.4781762, 1)	conservative
S. lewini	100000	1	(0.4781762, 1)	conservative
S. lewini	10000000		$(0.4781762\ 1)$	conservative

p = 0.90

Table 9: Mean proportion of observed unique haplotypes found over 50 replications of the simulation study for hypothetical species. In all cases, HACSim was run 100 times. Estimates correspond to the solid red horizontal lines depicted in confidence interval plots found within **Supplemental Information** accompanying the present article.

Scenario	Pop. size	R Mean	R Range	R Std. dev.
II	1000	0.9053333	0.852-0.948	0.01919418
II	10000	0.9193939	0.870 - 0.962	0.02000303
II	100000	0.9129091	0.864 - 0.946	0.01890166
II	10000000	0.9130303	0.864 - 0.940	0.01763605
III	1000	0.9468333	0.918 - 0.964	0.01410417
III	10000	0.9144167	0.874 - 0.950	0.02036176
III	100000	0.9148333	0.876 - 0.950	0.01983336
III	10000000	0.9115833	0.862 - 0.944	0.02228651
IV	1000	0.8660909	0.822 - 0.900	0.02006159
IV	10000	0.9093636	0.874 - 0.930	0.01783935
IV	100000	0.9183636	0.896 - 0.950	0.01355955
IV	10000000	0.9133636	0.866 - 0.940	0.01685102

Table 10: Mean proportion of observed unique haplotypes found over 50 replications of the simulation study for real species. In all cases, HACSim was run 100 times. Estimates correspond to the solid red horizontal lines depicted in confidence interval plots found within the Supplemental Information accompanying the present article.

Species	Pop. size	R Mean	R Range	R Std. dev.
Pea aphid (Acyrthosiphon pisum)	1000	0.8937446	0.850-0.915	0.01309869
Pea aphid ($Acyrthosiphon\ pisum$)	10000	0.8947836	0.8266667 - 0.9466667	0.02516662
Pea aphid $(Acyrthosiphon\ pisum)$	100000	0.9008658	0.828333 - 0.9466667	0.02355101
Pea aphid $(Acyrthosiphon\ pisum)$	10000000	0.9051732	0.8316667 - 0.9466667	0.02243506
Lake whitefish (Coregonus clupeaformis)	1000	0.9493771	0.9093333 - 0.9746667	0.01565928
Lake whitefish (Coregonus clupeaformis)	10000	0.9177268	0.8760000-0.9506667	0.01989952
Lake whitefish (Coregonus clupeaformis)	100000	0.9018798	0.8466667 - 0.9426667	0.02272721
Lake whitefish (Coregonus clupeaformis)	10000000	0.9094645	0.860 - 0.944	0.01890594
Common mosquito ($Culex\ pipiens$)	1000	0.9339833	0.9032 - 0.9592	0.01333865
Common mosquito (Culex pipiens)	10000	0.9128167	0.8832 - 0.9456	0.01534088
Common mosquito ($Culex\ pipiens$)	100000	0.9114667	0.8896 - 0.9416	0.01427876
Common mosquito (Culex pipiens)	10000000	0.9107833	0.8744 - 0.9448	0.01589668
Deer tick (Ixodes scapularis)	1000	0.9436145	0.9327711 - 0.9583133	0.008038651
Deer tick $(Ixodes\ scapularis)$	10000	0.9142972	0.9007229 - 0.9310843	0.008461128
Deer tick (Ixodes scapularis)	100000	0.9112115	0.8971084 - 0.9281928	0.00893221
Deer tick (Ixodes scapularis)	10000000	0.9110375	0.8978313 - 0.9265060	0.00824903
Gypsy moth $(Lymantria\ dispar)$	1000	0.9213282	0.9017241-0.94	0.01130016
Gypsy moth ($Lymantria\ dispar$)	10000	0.909387	0.8865517 - 0.9313793	0.01087714
Gypsy moth $(Lymantria\ dispar)$	100000	0.9100894	0.8903448 - 0.9320690	0.01193619
Gypsy moth $(Lymantria\ dispar)$	10000000	0.9108174	0.8868966 - 0.9306897	0.01150925
Scalloped hammerhead shark (Sphyrna lewini)	1000	0.935754	0.8966667-0.96	0.01433344
Scalloped hammerhead shark (Sphyrna lewini)	10000	0.8940873	0.8566667 - 0.9366667	0.01773106
Scalloped hammerhead shark (Sphyrna lewini)	100000	0.9107937	0.86 - 0.95	0.02057848
Scalloped hammerhead shark (Sphyrna lewini)	10000000	0.9073016	0.8533333-0.9483333	0.01848955

Table 11: Bonferroni-corrected Dunn's post-hoc test results at the 1.67% significance level for comparison of population size *versus* haplotype frequency distribution in hypothetical species. **Note**: KW test statistics, *p*-values and statistical significance for Scenario I could not be calculated.

Scenario	Pop. size pairing	Test statistic	<i>p</i> -value	Significant?
II	1000/10000	-3.091851	0.0060	Yes
II	1000/100000	-1.849633	0.1931	No
II	1000/10000000	-1.703015	0.2657	No
II	10000/100000	1.242218	0.6425	No
II	10000/10000000	1.388835	0.4946	No
II	100000/10000000	0.146617	1	No
III	1000/10000	4.826797	0	Yes
III	1000/100000	4.803466	0	Yes
III	1000/10000000	5.104169	0	Yes
III	10000/100000	-0.023330	1	No
III	10000/10000000	0.277372	1	No
III	100000/10000000	0.300702	1	No
IV	1000/10000	-4.830426	0	Yes
IV	1000/100000	-6.043938	0	Yes
IV	1000/10000000	-5.317602	0	Yes
IV	10000/100000	-1.213511	0.6748	No
IV	10000/10000000	-0.487176	1	No
IV	100000/10000000	0.726335	1	No

Table 12: Bonferroni-corrected Dunn's post-hoc test results at the 1.67% significance level for comparison of population size versus haplotype frequency distribution in real species.

Species	Pop. size pairing	Test statistic	<i>p</i> -value	Significant?
A. pisum	1000/10000	-1.031040	0.9076	No
A. pisum	1000/100000	-2.658617	0.0235	No
A. pisum	1000/10000000	-3.600946	0.0010	Yes
A. pisum	10000/100000	-1.627577	0.3108	No
A. pisum	10000/10000000	-2.569906	0.0305	No
A. pisum	100000/10000000	-0.942329	1	No
C. clupeaformis	1000/10000	6.719296	0	Yes
C. clupeaformis	1000/100000	9.849622	0	Yes
C. clupeaformis	1000/10000000	8.381319	0	Yes
C. clupeaformis	10000/100000	3.130326	0.0052	Yes
C. clupeaformis	10000/10000000	1.662023	0.2895	No
C. clupeaformis	100000/10000000	-1.468302	0.4261	No
C. pipiens	1000/10000	5.671734	0	Yes
C. pipiens	1000/100000	6.183339	0	Yes
C. pipiens	1000/10000000	6.301825	0	Yes
C. pipiens	10000/100000	0.511604	1	No
C. pipiens	10000/10000000	0.630090	1	No
C. pipiens	100000/10000000	0.118486	1	No
I. scapularis	1000/10000	6.461802	0	Yes
I. scapularis	1000/100000	7.737492	0	Yes
I. scapularis	1000/10000000	7.771397	0	Yes
I. scapularis	10000/100000	1.275690	0.6062	No
I. scapularis	10000/10000000	1.309595	0.5710	No
I. scapularis	100000/10000000	0.033905	1	No
$L.\ dispar$	1000/10000	4.818699	0	Yes
$L.\ dispar$	1000/100000	4.442226	0	Yes
$L.\ dispar$	1000/10000000	4.101167	0.0001	Yes
$L.\ dispar$	10000/100000	-0.376472	1	No
$L.\ dispar$	10000/10000000	-0.717531	1	No
$L.\ dispar$	100000/10000000	-0.341058	1	No
$S. \ lewini$	1000/10000	8.326225	0	Yes
$S. \ lewini$	1000/100000	4.978232	0	Yes
S. lewini	1000/10000000	5.778205	0	Yes
S, lewini	10000/100000	-3.347993	0.0024	Yes
$S.\ lewini$	10000/10000000	-2.548020	0.0325	No
S. lewini	100000/10000000	0.799972	1	No

 Table 13:
 Characteristics of local optima found by HACSim for hypothetical species.

0	NT.	NT*	AT*	TT:1 7 1 11	11: -14 1 - C.
Scenario	No. opuma	w range	IN Sta. dev.	rignest mode(s)	optima iv range iv stat dev. Highest mode(s) Highest mode ireq.
II	33	181-231	11.72283	202	10
III	24	155 - 199	7.704361	175	17
\sim	22	123-158	7,886339	139	

Table 14: Characteristics of local optima found by HACSim for real species. For some species, distinct optima were located for different population sizes.

Species	No. optima	N^* range	N^* std. dev.	Highest mode(s)	optima N^* range N^* std. dev. Highest mode(s) Highest mode freq.
A. pisum	22	623-962	65.23966	777/788/837	3
$C.\ clupe a form is$	61	383-542	34.27441	462	v
C. pipiens	48	424-549	27.83662	446	~
$I.\ scapularis$	36	574-665	25.14735	588	11
$L. \ dispar$	54	651 - 765	39.27425	672	rc
S. lewini	42	244-343	19.29278	279	6

Table 15: Coverage probabilities and 95% Clopper-Pearson confidence intervals for hypothetical species for p = 0.90 across all assessed population sizes. Calculated probabilities are based on the number of observed local optima found using HACSim.

Scenario	Pop. size	Coverage prob.	scenario Pop. size Coverage prob. 95% Exact binomial CI Coverage type	Coverage type
II	1000	1	(0.8942372, 1)	conservative
II	10000	1	(0.8942372, 1)	conserative
II	100000	П	(0.8942372, 1)	conservative
II	10000000	0.969697	(0.8424060, 0.9992331)	conservative
III	1000	1	(0.8575264, 1)	conservative
III	10000	П	(0.8575264, 1)	conservative
III	100000	1	(0.8575264, 1)	conservative
III	10000000	0.9166667	(0.7300272, 0.9897437)	conservative
IV	1000	П	(0.8456275, 1)	conservative
IV	10000	П	(0.8456275, 1)	conservative
IV	100000	1	(0.8456275, 1)	conservative
IV	10000000	1	(0.7715556, 0.9988499)	conservative

Table 16: Coverage probabilities and 95% Clopper-Pearson confidence intervals for real species for $\mathbf{p} = 0.90$ across all assessed population sizes. Calculated probabilities are based on the number of observed local optima found using HACSim.

Species	Pop. size	Coverage prob.	95% Exact binomial CI	Coverage type
A. pisum	1000		(0.9532219, 1)	conservative
A. pisum	10000	\vdash	(0.9532219, 1)	conservative
A. pisum	100000		(0.9532219, 1)	conservative
A. pisum	10000000	0.9220779	(0.8380736, 0.9708670)	conservative
$C.\ clupe a form is$	1000	1	(0.9413188, 1)	conservative
$C.\ clupe a form is$	10000	П	(0.94131881)	conservative
$C.\ clupe a form is$	100000	1	(0.9413188, 1)	conservative
$C.\ clupe a form is$	10000000	0.9016393	(0.7981038, 0.9630400)	conservative
$C.\ pipiens$	1000	П	(0.9260272, 1)	conservative
$C.\ pipiens$	10000	\vdash	(0.9260272, 1)	conservative
$C.\ pipiens$	100000		(0.9260272, 1)	conservative
$C.\ pipiens$	10000000	0.9583333	(0.8574590, 0.9949135)	conservative
$I.\ scapularis$	1000	П	(0.9026062, 1)	conservative
$I.\ scapularis$	10000	\vdash	(0.9026062, 1)	conservative
$I.\ scapularis$	100000		(0.9026062, 1)	conservative
$I.\ scapularis$	10000000	\vdash	(0.9026062, 1)	conservative
L. dispar	1000	\vdash	(0.9339685, 1)	conservative
L. dispar	10000		(0.9339685, 1)	conservative
L. dispar	100000		(0.9339685, 1)	conservative
L. dispar	10000000	0.962963	(0.8725282, 0.9954827)	conservative
S. lewini	1000	\vdash	(0.9159161, 1)	conservative
S. lewini	10000	\vdash	(0.9159161, 1)	conservative
S. lewini	100000	\vdash	(0.9159161, 1)	conservative
S. lewini	10000000	0.9761905	(0.8743414, 0.9993974)	conservative

p = 0.95

Table 17: Mean proportion of observed unique haplotypes found over 50 replications of the simulation study for hypothetical species. In all cases, HACSim was run 100 times. Estimates correspond to the solid red horizontal lines depicted in confidence interval plots found within Figures 13-15 and Supplemental Information accompanying this paper.

Scenario	Pop. size	R Mean	R Range	R Std. dev.
II	1000	0.962	0.936-0.980	0.009999
II	10000	0.956	0.926 - 0.976	0.0126
II	100000	0.953	0.914 - 0.978	0.0152
II	10000000	0.954	0.918 - 0.978	0.0125
III	1000	0.934	0.886 - 0.970	0.0148
III	10000	0.954	0.922 - 0.976	0.0130
III	100000	0.951	0.924 - 0.978	0.0137
III	10000000	0.955	0.930 - 0.970	0.0102
IV	1000	0.965	0.940 - 0.988	0.0117
IV	10000	0.960	0.942 - 0.978	0.00926
IV	100000	0.954	0.930 - 0.978	0.0117
IV	10000000	0.954	0.936 - 0.980	0.0128

Table 18: Mean proportion of observed unique haplotypes found over 50 replications of the simulation study for real species. In all cases, HACSim was run 100 times. Estimates correspond to the solid red horizontal lines depicted in confidence interval plots found within Figures 14-21 and Supplemental Information accompanying this paper.

Species	Pop. size	R Mean	R Range	R Std. dev.
Pea aphid (Acyrthosiphon pisum)	1000	0.999	0.992-1	0.00212
Pea aphid $(Acyrthosiphon\ pisum)$	10000	0.954	0.928 - 0.990	0.0114
Pea aphid $(Acyrthosiphon\ pisum)$	100000	0.955	0.920 - 0.980	0.0118
Pea aphid $(Acyrthosiphon\ pisum)$	10000000	0.951	0.920 - 0.977	0.0130
Common mosquito (Culex pipiens)	1000	0.956	0.926 - 0.974	0.00913
Common mosquito (Culex pipiens)	10000	0.958	0.925 - 0.974	0.00903
Common mosquito (Culex pipiens)	100000	0.951	0.934 - 0.972	0.00780
Common mosquito (Culex pipiens)	10000000	0.952	0.931 - 0.968	0.00824
Deer tick (Ixodes scapularis)	1000	0.940	0.926 - 0.953	0.00622
Deer tick $(Ixodes\ scapularis)$	10000	0.954	0.942 - 0.965	0.00579
Deer tick $(Ixodes\ scapularis)$	100000	0.954	0.936 - 0.965	0.00620
Deer tick $(Ixodes\ scapularis)$	10000000	0.954	0.938 - 0.970	0.00595
Gypsy moth ($Lymantria\ dispar$)	1000	0.937	0.918 - 0.947	0.00658
Gypsy moth ($Lymantria\ dispar$)	10000	0.956	0.933 - 0.972	0.00844
Gypsy moth ($Lymantria\ dispar$)	100000	0.952	0.934 - 0.968	0.00762
Gypsy moth ($Lymantria\ dispar$)	10000000	0.952	0.932 - 0.966	0.00814
Scalloped hammerhead shark (Sphyrna lewini)	1000	0.965	0.938 - 0.988	0.00981
Scalloped hammerhead shark (Sphyrna lewini)	10000	0.945	0.912 - 0.973	0.0128
Scalloped hammerhead shark (Sphyrna lewini)	100000	0.949	0.917 - 0.975	0.0148
Scalloped hammerhead shark (Sphyrna lewini)	10000000	0.949	0.923 - 0.972	0.0127

Table 19: Bonferroni-corrected Dunn's post-hoc test results at the 0.83% significance level for comparison of population size *versus* haplotype frequency distribution in hypothetical species. **Note**: KW test statistics, *p*-values and statistical significance for Scenario I could not be calculated.

Scenario	Pop. size pairing	Test statistic	<i>p</i> -value	Significant?
II	1000/10000	2.322	0.061	No
II	1000/100000	3.270	0.00320	Yes
II	1000/10000000	3.22	0.00380	Yes
II	10000/100000	0.948	1	No
II	10000/10000000	0.900	1	No
II	100000/10000000	-0.0486	1	No
III	1000/10000	-5.396	0	Yes
III	1000/100000	-4.307	0	Yes
III	1000/10000000	-5.698	0	Yes
III	10000/100000	1.089	0.828	No
III	10000/10000000	-0.303	1	No
III	100000/10000000	-1.392	0.492	No
IV	1000/10000	1.666	0.287	No
IV	1000/100000	3.358	0.00240	Yes
IV	1000/10000000	3.159	0.00480	Yes
IV	10000/100000	1.692	0.272	No
IV	10000/10000000	1.492	0.407	No
IV	100000/10000000	-0.1995	1	No

Table 20: Bonferroni-corrected Dunn's post-hoc test results at the 0.83% significance level for comparison of population size *versus* haplotype frequency distribution in real species.

Species	Pop. size pairing	Test statistic	<i>p</i> -value	Significant?
A. pisum	1000/10000	8.506	0	Yes
A. pisum	1000/100000	8.018	0	Yes
A. pisum	1000/10000000	9.348	0	Yes
A. pisum	10000/100000	-0.619	1	No
A. pisum	10000/10000000	1.068	0.8560	No
A. pisum	100000/10000000	1.688	0.275	No
C. pipiens	1000/10000	-1.341	0.540	No
C. pipiens	1000/100000	3.475	0.00150	Yes
C. pipiens	1000/10000000	2.841	0.0135	No
C. pipiens	10000/100000	4.816	0	Yes
C. pipiens	10000/10000000	4.182	0.000100	Yes
C. pipiens	100000/10000000	-0.634	1	No
I. scapularis	1000/10000	-8.204	0	Yes
I. scapularis	1000/100000	-8.856	0	Yes
I. scapularis	1000/10000000	-8.050	0	Yes
I. scapularis	10000/100000	-0.649	1	No
I. scapularis	10000/10000000	0.154	1	No
I. scapularis	100000/10000000	0.803	1	No
$L.\ dispar$	1000/10000	-10.411	0	Yes
$L.\ dispar$	1000/100000	-7.584	0	Yes
$L.\ dispar$	1000/10000000	-7.928	0	Yes
$L.\ dispar$	10000/100000	2.905	0.011	No
$L.\ dispar$	10000/10000000	2.551	0.0322	No
$L.\ dispar$	100000/10000000	-0.354	1	No
S. lewini	1000/10000	7.901	0	Yes
$S.\ lewini$	1000/100000	5.983	0	Yes
S. lewini	1000/10000000	6.409	0	Yes
S, lewini	10000/100000	-1.918	0.165	No
S. lewini	10000/10000000	-1.492	0.407	No
$S.\ lewini$	100000/10000000	0.426	1	No

 Table 21:
 Characteristics of local optima found by HACSim for hypothetical species.

N* C+d dow High
N^* range N^* Std. dev. Highest mode(s) Highest mode freq.
226-296 13.469
200-252 11.009
176-207 8.104

Table 22: Characteristics of local optima found by HACSim for real species. For some species, distinct optima were located for different population sizes. * corresponds to a population size of 1000 and ** to population sizes of 10000, 100000 and 10000000.

${f Species}$	No. optima	N^* range	N^* Std. dev.	Highest mode(s)	N^* Std. dev. Highest mode(s) Highest mode freq.
A. pisum	**08/*98	868-999*/868-1230** 33.677*/68.380**	33.677*/68.380**	994	4
$C.\ pipiens$	27	529-666	25.128	579	9
I. scapularis	09	744-896	32.939	808	ಬ
L. dispar	L. dispar $63*/70**$	816-985*/816-1048**	$42.434^*/48.938^{**}$	909/917/921	4
S. lewini	65	329-458	26.395	400	4

Table 23: Coverage probabilities and 95% Clopper-Pearson confidence intervals for hypothetical species across all assessed population sizes. Calculated probabilities are based on the number of observed local optima found using HACSim.

Scenario	Pop. size	cenario Pop. size Coverage prob.	95% Exact binomial CI Coverage type	Coverage type
II	1000	1	(0.921, 1)	conservative
II	10000	0.978	(0.882, 0.999)	conservative
II	100000	0.956	(0.849, 0.995)	conservative
II	10000000	0.956	(0.849, 0.995)	conservative
III	1000	0.658	(0.486, 0.804)	permissive
III	10000	0.974	(0.862, 0.999)	conservative
III	100000	0.974	(0.862, 0.999)	conservative
III	10000000	0.974	(0.862, 0.999)	conservative
Λ	1000	П	(0.881, 1)	conservative
IV	10000		(0.881, 1)	conservative
IV	100000		(0.881, 1)	conservative
IV	10000000	1	(0.881, 1)	conservative

Table 24: Coverage probabilities and 95% Clopper-Pearson confidence intervals for real species across all assessed population sizes. Calculated probabilities are based on the number of observed local optima found using HACSim.

Species	Pop. size	Coverage prob.	95% Exact binomial CI Coverage type	Coverage type
A. pisum	1000	П	(0.903, 1)	conservative
A. pisum	10000	0.975	(0.913, 0.997)	conservative
A. pisum	100000	0.975	(0.913, 0.997)	conservative
A. pisum	100000001	0.925	(0.844, 0.972)	permissive
$C.\ pipiens$	1000	0.965	(0.879, 0.996)	conservative
$C.\ pipiens$	10000	0.982	(0.906, 1)	conservative
$C.\ pipiens$	100000	0.930	(0.830, 0.981)	permissive
$C.\ pipiens$	10000000	0.965	(0.879, 0.996)	conservative
$I.\ scapularis$	1000	0.150	(0.0710, 0.266)	permissive
$I.\ scapularis$	10000	0.983	(0.911, 1)	conservative
$I.\ scapularis$	100000	0.900	(0.795, 0.962)	permissive
$I.\ scapularis$	100000001	0.950	(0.861, 0.990)	exact
L. dispar	1000	0	(0, 0.0569)	permissive
L. dispar	10000	0.943	(0.860, 0.984)	permissive
L. dispar	100000	0.900	(0.805, 0.959)	permissive
L. dispar	10000000	0.843	(0.736, 0.919)	permissive
S. lewini	1000	П	(0.945, 1)	conservative
S. lewini	10000	0.846	(0.735, 0.924)	permissive
S. lewini	100000	0.877	(0.772, 0.945)	permissive
S. $lewini$	10000000	0.908	(0.810, 0.965)	permissive