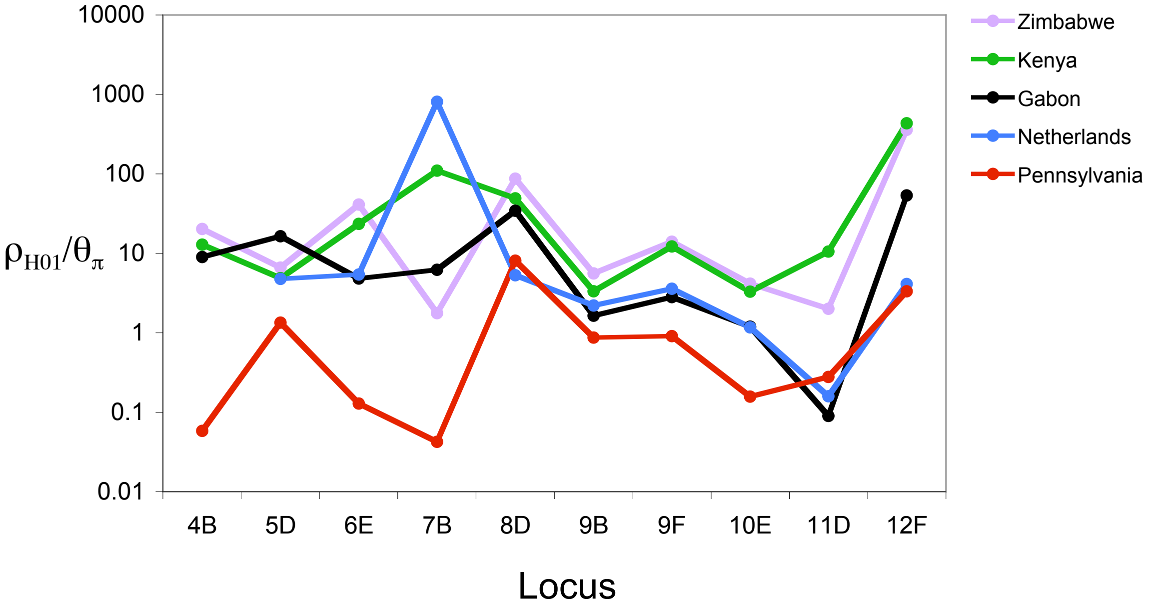
Supplementary Results 1: Summary of nucleotide variability by locus and population.

Locus/Pop	Cyt	L	n	S	θ_W	π	D	H	K	R_M
Fasciclin -2	4B	531								
Zimbabwe			17	18	0.0128	0.0110	-0.54	1.55	14	3
Kenya			21	15	0.0099	0.0078	-0.76	-0.01	13	2
Gabon			22	20	0.0129	0.0105	-0.69	1.51	13	5
Netherlands			21	0	0.0000	0.0000	-	-	1	-
Pennsylvania			21	8	0.0052	0.0018	-2.19	-3.08	3	0
spaghetti squash	5E	549								
Zimbabwe			16	18	0.0107	0.0097	-0.35	2.82	11	2
Kenya			24	29	0.0153	0.0128	-0.61	1.25	18	4
Gabon			21	26	0.0143	0.0111	-0.85	3.36	17	3
Netherlands			20	12	0.0067	0.0045	-1.15	-0.98	7	1
Pennsylvania			21	16	0.0088	0.0065	-0.95	0.69	9	1
CG32732	6E	503								
Zimbabwe			17	17	0.0121	0.0088	-1.06	0.82	17	3
Kenya			20	24	0.0165	0.0122	-0.99	-3.43	17	3
Gabon			20	20	0.0140	0.0101	-1.04	0.52	15	2
Netherlands			19	6	0.0042	0.0035	-0.52	-3.01	5	1
Pennsylvania			23	2	0.0013	0.0008	-0.86	-1.43	2	0
cut	7B	489								
Zimbabwe			20	14	0.0086	0.0060	-1.09	-2.25	6	0
Kenya			20	7	0.0043	0.0029	-1.06	0.36	8	1
Gabon			21	5	0.0030	0.0010	-1.98	0.45	5	0
Netherlands			21	3	0.0018	0.0010	-1.22	0.40	4	0
Pennsylvania			22	8	0.0048	0.0025	-1.58	-4.10	4	0
lozenge	8D	536								
Zimbabwe			20	19	0.0117	0.0111	-0.19	-1.29	18	8
Kenya			22	20	0.0120	0.0097	-0.71	-1.40	19	3
Gabon			20	18	0.0115	0.0088	-0.88	-2.97	18	4
Netherlands			18	10	0.0066	0.0089	1.28	1.41	7	3
Pennsylvania			19	9	0.0056	0.0075	1.19	0.12	7	3
Hyperkinetic	9B	548								
Zimbabwe			21	46	0.0352	0.0386	0.38	3.80	18	8
Kenya			24	40	0.0287	0.0317	0.40	0.24	22	6
Gabon			21	40	0.0301	0.0309	0.10	-3.52	13	7
Netherlands			21	26	0.0178	0.0121	-1.21	-9.81	5	1
Pennsylvania			22	20	0.0134	0.0085	-1.37	-10.60	3	0
vermilion	9F	565								
Zimbabwe			21	20	0.0118	0.0136	0.59	2.08	17	5
Kenya			23	23	0.0131	0.0141	0.30	2.00	20	8
Gabon			18	24	0.0150	0.0120	-1.27	-5.46	14	2

Netherlands			21	9	0.0053	0.0043	-0.65	-6.66	6	1
Pennsylvania			22	14	0.0081	0.0096	0.67	-3.17	7	2
dusky	10E	531								
Zimbabwe			20	40	0.0253	0.0310	0.88	2.08	16	8
Kenya			18	41	0.0268	0.0294	0.39	1.86	14	9
Gabon			21	39	0.0239	0.0258	0.32	-1.73	13	3
Netherlands			22	8	0.0048	0.0020	-1.95	-6.60	4	0
Pennsylvania			23	24	0.0143	0.0147	0.10	-7.84	5	0
licorne	11D	546								
Zimbabwe			23	18	0.0121	0.0085	-1.09	0.47	14	2
Kenya			20	11	0.0085	0.0088	0.11	1.14	12	3
Gabon			18	5	0.0036	0.0033	-0.30	0.39	4	0
Netherlands			23	4	0.0026	0.0016	-1.07	-4.58	2	0
Pennsylvania			21	3	0.0020	0.0007	-1.73	-5.43	2	0
rutabaga	12F	505								
Zimbabwe			22	7	0.0050	0.0029	-1.36	-2.27	9	1
Kenya			23	8	0.0057	0.0024	-1.88	-0.55	8	0
Gabon			23	8	0.0057	0.0041	-0.93	-0.17	9	1
Netherlands			18	6	0.0045	0.0035	-0.73	-0.58	6	0
Pennsylvania			20	4	0.0029	0.0031	0.20	-0.43	5	0

Note. – Cyt = cytological location; *L* = length of sequenced region in base pairs; *n* = sample size; *S* = number of polymorphic sites; θ_W = Watterson's (1975) diversity estimate; π = average pairwise divergence (Tajima 1983); *D* = Tajima's (1989) *D* test statistic; *H* = Fay and Wu's (2000) *H* test statistic; *K* = Number of distinct haplotypes; R_M = minimum number of inferred recombination events (Hudson and Kaplan. 1985). Diversity estimates are per site.

Supplementary Results 2. Per locus estimates of ρ/θ by population. ρ was estimated using Hudson's (2001) estimator. θ was estimated as π, the average pairwise diversity per site.



All ten estimates of ρ/θ (measured as ρ_{H01}/θ_{π}, see Methods) for the Pennsylvania population are below those of Kenya and Zimbabwe and nine of ten are lower than in Gabon ($P = 0.002$ and $P = 0.022$, by two-tailed Sign tests, respectively). The same trend is apparent in the Netherlands population, though this was not significant. Nine of the ten estimates of ρ/θ in the Gabon population are lower than the average of the estimates for Kenya and Zimbabwe (Figure 4, $P = 0.022$ by a two-tailed Sign test).

Supplementary Results 3. Quantifying the effect of mis-inferring the ancestral state when calculating Fay and Wu's *H*.

A crude "back of the envelope" way of addressing this issue is the following (see also Fay and Wu 2000). The chance of misinferring an ancestral state requires that a back-mutation occur on the *D. simulans* branch; this has a probability of roughly the length of that branch (Dxy/2). There are three possible back-mutations, but only 1/3 of them will lead us to misinfer the ancestral state. Thus the probability that we misinfer the ancestral state is roughly equal to Dxy/6. We have implemented a correction based on these calculations and have repeated the simulations in Table 4. The new expectations and *P*-values for the Fay and Wu's *H* column in Table 4 would be as follows:

Model	Mean *H*
SNM	-0.14
	(0.065)
G1	0.23
	($<10^{-4}$)
G2	0.73
	($<10^{-4}$)
HH1	0.31
	(10^{-3})
HH2	0.30
	(10^{-3})
BN	-0.39
	(0.405)

As expected, occasionally misinferring the ancestral state does lead to a slightly more negative Fay and Wu's *H* than expected under the infinite sites model. However, incorporating this detail only changes the quantitative but not the qualitative conclusions of the paper. The observed mean *H* in Zimbabwe still looks somewhat unusually negative under the standard neutral model (though the standard neutral model has already been rejected based on the mean Tajima's *D* and the multilocus HKA test), but the main point is that the mean *H* is not compatible with population growth and hitchhiking models (both conclusions that are not affected by this correction). For the simulations done in Tables 3 and 5, the correction also does not change any of the conclusions.