The following supplements accompany the article

Population genetic structure in European lobsters: implications for connectivity, diversity and hatchery stocking

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Supplement 1. Technical and explanatory details of methodologies further to that provided in the main article.

Ethics statement

Permission to obtain lobster tissue samples from Cornwall and the Isles of Scilly was obtained from the fishery regulators and managers; the Inshore Fisheries Conservation Authorities of Cornwall and Scilly within coastal waters (<6 nmi.), and the Marine Management Organisation within offshore waters (>6 nmi.). Samples from these locations were collected in situ on board commercial vessels as part of regular fishing routines, with permission granted to allow the temporary holding (for sampling) of individuals normally in breach of regional bylaws (Cornwall IFCA, www.cornwall-ifca.gov.uk). Elsewhere, lobster tissue samples or extracted DNA were obtained from landed individuals comprising the legal catch, requiring only the permission of the owning merchants. All tissue sampling was non-lethal and involved no endangered or protected species; the European lobster is categorised as being of Least Concern in the Red List of Threatened Species of the International Union for Conservation of Nature (Butler et al., 2015).

Statistical analysis

The inbreeding coefficient F_{IS} (Weir & Cockerham, 1984) was used by GENEPOP (Raymond & Rousset, 1995a) to check Hardy-Weinberg equilibrium (HWE). LOSITAN (Antao et al., 2008) utilises the Fdist method (Beaumont & Nichols, 1996) to test loci for signatures of selection. FSTAT (Goudet, 2001) obtained the standard error of global F_{ST} estimates by jackknifing over loci, and 95% confidence intervals via 15,000 bootstraps over loci. *G* tests (Goudet et al., 1996) to obtain *p*-values for global and pairwise F_{ST} estimates were obtained via 50,000 and >7,500 permutations, respectively, conducted in FSTAT. *G* tests were preferred to the alternative Fisher's exact test because they weight results according to the polymorphism of loci, and so provide a more accurate and conservative measure of significance for multi-locus data with low levels differentiation (Goudet, 2001; Petit et al., 2001; Ryman et al., 2006). However, F_{ST} *p*-values were also estimated via exact tests (conducted in GENEPOP – Raymond & Rousset, 1995b), to allow comparison with results from POWSIM, which estimates F_{ST} *p*-values via exact tests but not *G* tests (Ryman & Palm, 2006).

G tests (Goudet et al., 1996) in DEMEtics (Gerlach et al., 2010) used 1,000 bootstraps to provide 95% confidence intervals and *p*-values for global and per-locus estimates of Jost's *D* (Jost, 2008). Benjamini & Yekutieli's (2001) modified false discovery rate (FDR) method was chosen to adjust the significance threshold for pairwise F_{ST} *p*-values because it better controls Type I (α) error than the original FDR approach of Benjamini & Hochberg (1995) without the loss of power to distinguish meaningful genetic differentiation that occurs with the overly conservative Bonferroni correction (Narum, 2006).

Minimum oceanic distances between geographic samples were obtained using Free Map Tools (www.freemaptools.com), and isolation by distance tested using ISOLDE function (Rousset, 1997) in GENEPOP. NeEstimator (Do et al., 2014) used the LD method (Waples, 2006; Waples & Do, 2008) to estimate effective population sizes (N_e). N_e was measured for each geographic sample, although, because its estimation assumes populations are closed and non-continuous distributed (Waples & England, 2011; Neel et al., 2013), results are generally unreliable when the spatial definition of

populations and other demographic parameters are not already established (Wang, 2005; Neel et al., 2013).

The LOCPRIOR setting used in STRUCTURE (Pritchard et al., 2000) effectively informs the model of which individuals constitute each spatial sample (i.e. basic sample groupings, rather than explicit data on spatial position or relative distances), and instead of an assumption that all possible partitions of K are equally likely, the clustering algorithm is therefore able to assert greater weight to assignments which correlate with sample groupings (Hubisz *et al*., 2009). This improves the detection of population divergence but does not infer it when it is absent, since algorithms ignore the designation of samples where no correlations exist with genotype clusters (Hubisz et al., 2009). The analysis of molecular variance (AMOVA) conducted in ARLEQUIN (Excoffier & Lischer, 2010) used >16,000 permutations and was weighted by locus to account for missing data. Because groups of geographic samples reflected the cluster assignments inferred by Bayesian, AMOVA significance tests were ignored since these are biased by circularity (Meirmans, 2015), as well as by the confounding effects of IBD (Meirmans, 2012).

Analysis of Power

In POWSIM analyses (Ryman & Palm, 2006), Fisher's exact tests were used to examine genetic differentiation between subsamples because it provides a more stable estimator of α error and power than the alternative chi-square test, particularly when assessing multi-locus genotypes with skewed allele frequencies (Ryman et al., 2006). POWSIM computations require an estimate of N_e for the base population, which controls the generations of drift required to attain an expected level of differentiation, but which has a negligible effect on the statistical power obtained at that F_{ST}. We compared two estimates of N_e. The lower estimate was 2000, close to that estimated for the large Strömstad sample which should be low for the species as a result of historic overfishing (Vucetich et al., 1997; Kalinowski & Waples, 2002; Huserbråten et al., 2013). An upper N_e of 10000 was tested for comparison, which was based on a typical N_e / N_{CENSUS} of 0.005 for highly fecund marine species (Frankham, 1995; Turner et al., 2002; Ovenden et al., 2007; Palstra & Fraser, 2012) and calculated via the estimated stock size for the Cornwall region (CEFAS, 2015). The detection of overall differentiation featured 5000 subsample replicates per simulation of drift, and for pairwise differentiation it followed 1000 subsample replicates.

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Supplement 2. Tables and figures

Table S1. Global descriptive statistics of microsatellite loci. The total number of alleles (N_A), *p*-value of test for Hardy-Weinberg equilibrium (HW *p*), total heterozygosity (H_T), and two measures of differentiation; the fixation index (F_{ST}) and Jost's differentiation (*D*), with associated confidence intervals (95% C.I.) or standard error [s.e.], and *p*-values derived from Fisher's exact test (F_{ST} *p*) or 1000 bootstrap replicates (*D p*).

Locus	Genbank accession	N_A	HW *p*	H_T	F_{ST} [s.e.]	F_{ST} *p*	*D* (95% C.I.)	*D p*
HGD106	GU233670	12	0.833	0.715	–0.001 [0.004]	0.08	0.011 (–0.018-0.048)	0.01
HGC118	GU233666	9	0.819	0.583	0.008 [0.005]	0.00	0.017 (–0.002-0.041)	0.00
HGB4	GU233661	12	0.548	0.612	0.006 [0.006]	0.31	0.028 (0.008-0.055)	0.01
HGD117	KT240104	12	0.519	0.569	0.003 [0.006]	0.03	0.005 (–0.027-0.048)	0.43
HGC103	GU233664	9	0.707	0.693	0.019 [0.009]	0.00	0.057 (0.022-0.095)	0.07
HGB6	GU233662	11	0.951	0.737	0.034 [0.010]	0.00	0.064 (0.028-0.107)	0.76
HGD129	KT240105	11	0.935	0.556	0.004 [0.007]	0.40	0.024 (0.007-0.046)	0.79
HGC6	GU233663	8	0.989	0.408	0.013 [0.009]	0.01	0.015 (0.007-0.026)	0.91
HGC129	GU233668	14	0.207	0.754	0.002 [0.006]	0.05	–0.006 (–0.068-0.062)	0.01
HGC111	GU233665	11	0.216	0.732	0.018 [0.007]	0.00	0.002 (–0.032-0.044)	0.21
HGD111	GU233671	15	0.826	0.573	–0.005 [0.005]	0.51	0.007 (–0.013-0.031)	0.47
HGD110	KT240103	13	0.961	0.802	–0.001 [0.004]	0.18	–0.007 (–0.075-0.072)	0.27
HGC131b	GU233669	13	0.369	0.821	–0.003 [–0.002]	0.95	–0.036 (–0.085-0.016)	0.31
HGC120	GU233667	20	0.986	0.866	–0.003 [–0.003]	0.36	–0.026 (–0.083-0.033)	0.03
Overall					**0.007 [0.003]**		**0.011**	
	-	**170**	**0.998**	**0.673**		**0.00**		**0.01**
(95% C.I.)					**(0.002 - 0.012)**		**(0.000 - 0.023)**	

Table S2. **By-sample genetic variability.** Genetic variability data of geographic lobster samples, with Fig. 1 key and approximate location, the number of individuals (n), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}), allelic richness (A_R), p values of exact probability tests of Hardy-Weinberg disequilibrium (HW p), and effective size (N_e).

Map key	Geographical sample	n	H_O	H_E	F_{IS}	A_R	HW p	N_e
BS	Boscastle, UK	24	0.723	0.686	–0.056	3.738	0.699	60.2[a]
TT	Tintagel, UK	24	0.598	0.657	0.091	3.521	0.030	146.4[a]
PW	Padstow, UK	24	0.696	0.677	–0.029	3.690	0.435	203.4[a]
NQ	Newquay, UK	24	0.705	0.700	–0.008	3.809	0.577	∞
PT	Portreath, UK	24	0.696	0.687	–0.014	3.752	0.213	125.1[a]
HY	Hayle, UK	24	0.690	0.672	–0.028	3.657	0.729	529.5[a]
SN	Sennen, UK	24	0.655	0.678	0.034	3.730	0.128	762.3[a]
MZ	Marazion, UK	24	0.625	0.645	0.031	3.465	0.122	232.4[a]
LD	Lizard, UK	24	0.661	0.665	0.006	3.588	0.958	∞
FH	Falmouth, UK	24	0.658	0.669	0.016	3.669	0.479	579.9[a]
SA	St Austell, UK	24	0.616	0.637	0.034	3.561	0.916	168.0[a]
LO	Looe, UK	24	0.658	0.669	0.017	3.633	0.078	∞
SC	Scilly Isles, UK	24	0.673	0.684	0.017	3.764	0.670	620.2[a]
BR	Bergen, Norway	8	0.721	0.710	–0.019	3.668	0.904	∞
SV	Stavanger, Norway	8	0.609	0.650	0.070	3.486	0.996	∞
SD	Strömstad, Sweden	96	0.669	0.677	0.012	3.627	0.556	2406.9
LK	Lysekil, Sweden	96	0.715	0.705	–0.014	3.882	0.171	∞
HL	Helgoland, Germany	5	0.714	0.671	–0.072	3.580	1.000	30.9
OI	Orkney, UK	10	0.687	0.643	–0.073	3.566	0.986	∞
NH	Northumberland, UK	11	0.669	0.658	–0.017	3.474	0.640	22.0
NF	Norfolk, UK	8	0.680	0.707	0.041	3.769	0.514	∞
SX	Sussex, UK	9	0.619	0.651	0.052	3.596	0.731	∞
LY	Llyn, UK	10	0.611	0.647	0.060	3.498	0.315	∞
PM	Pembrokeshire, UK	10	0.629	0.656	0.043	3.646	0.967	248.2
GW	Galway, Ireland	7	0.663	0.662	–0.001	3.540	0.998	∞
LR	La Rochelle, France	7	0.609	0.638	0.054	3.199	0.995	∞
VG	Vigo, Spain	8	0.625	0.652	0.045	3.641	0.945	∞
LZ	Lazio, Italy	7	0.692	0.692	0.000	3.788	0.913	∞
Total / *weighted mean*		**612**	***0.672***	***0.676***	***0.007***	***3.674***	**0.998**	

[a] = N_e is infinite when Cornwall samples treated as a single population

Table S3 – Results of global AMOVA, as a weighted average of locus-by-locus tests. Atlantic (NF, SX, NH, OI, GW, LY, PM, BS, TT, PW, NQ, PT, HY, SN, SC, MZ, LD, FH, SA, LO, LR, VG & SV) and Eastern North Sea (LK, SD, HL, BR & LZ) sample groups were defined by majority assignment in cluster analysis.

Source of variation	Mean d.f.	Sum of squares	Variance components	Percentage variation	Fixation index
Among groups	1	30.00	0.05	1.02	0.008
Among populations within groups	26	132.79	0.01	0.20	0.002
Among individuals within populations	444	2,540.56	0.04	0.76	0.010
Within individuals	567	2,640.00	4.67	98.01	0.020
TOTAL	-	5,343.35	4.76	100.00	-

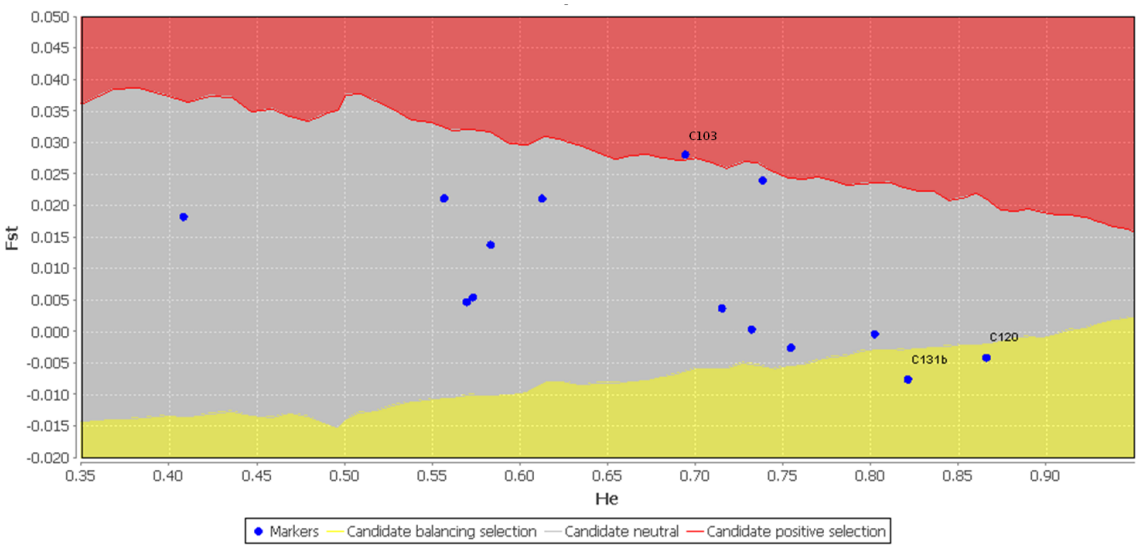

Fig. S1. Plot of markers under selection across all samples. LOSITAN plot of H_E vs F_{ST} for all samples and all markers. The grey zone denotes selective neutrality; markers (blue dots) falling into estimated regions of directional (red) and balancing (yellow) selection are labelled.

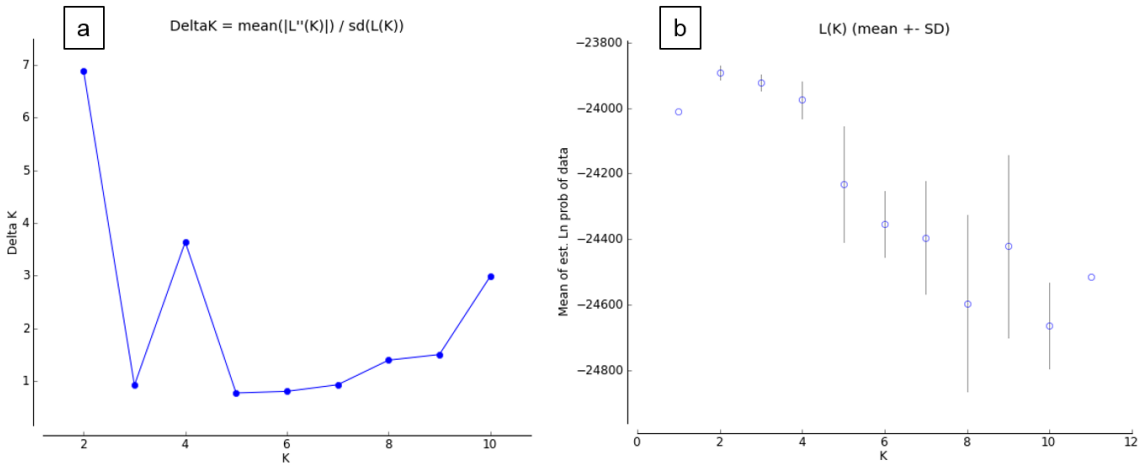

Fig. S2. Likelihood of the number of population clusters. Clockwise from top left: plots of cluster likelihood via **[a]** Evanno's delta-K and **[b]** the mean log likelihood.

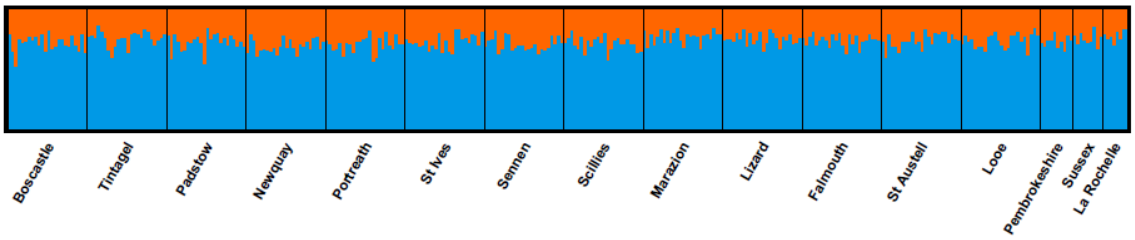

Fig. S3. Plot of fine-scale cluster assignment. Distruct plot of convergence of K = 2 for five iterations of the fine-scale dataset of samples from Cornwall, U.K., and nearby outgroups, from STRUCTURE models with *a priori* location data.

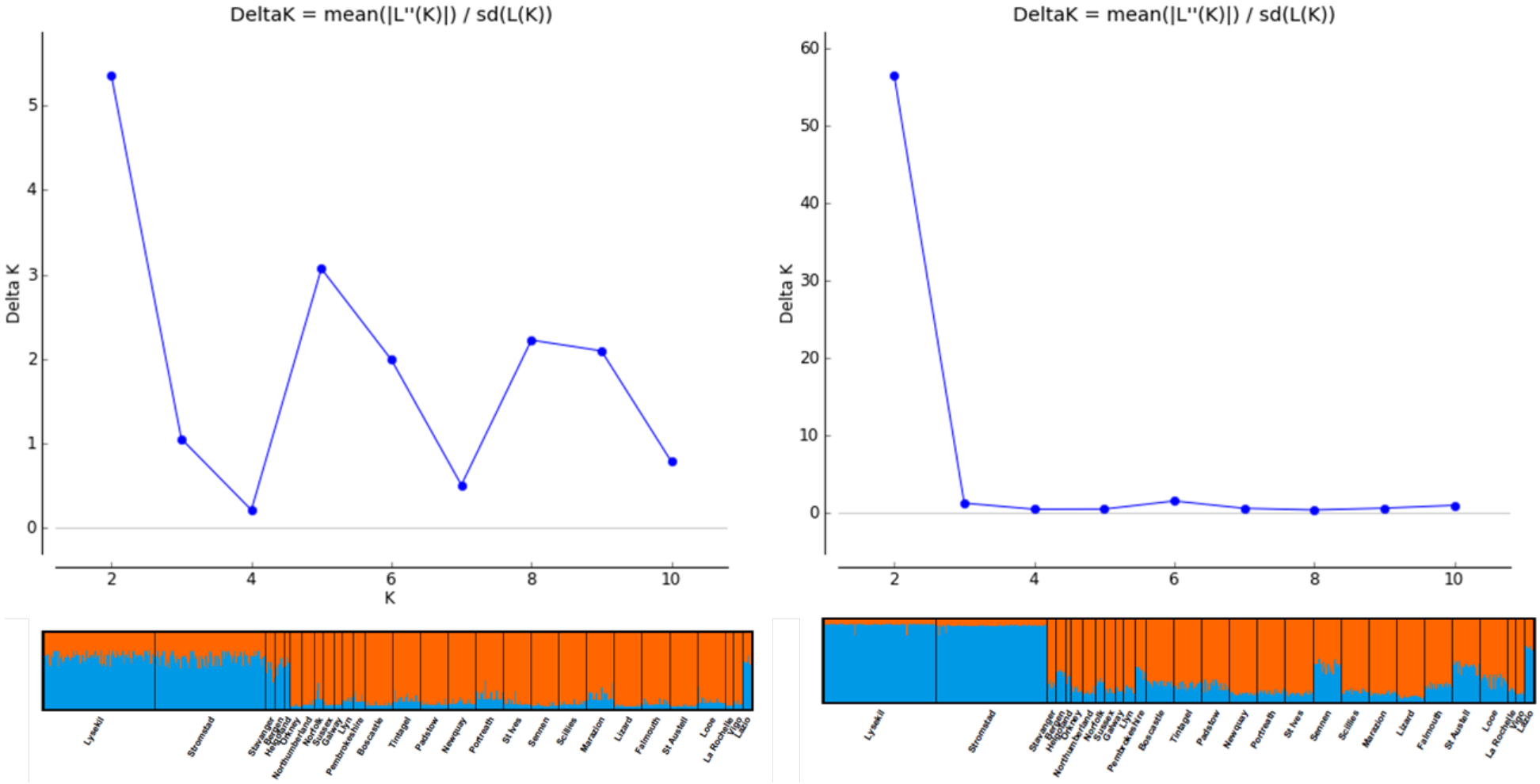

Fig. S4. Single-locus plots of cluster likelihood and population assignment. Plots of Evanno's delta-K (top) and Distruct plots of convergence (bottom; min. 5 iterations) from single-locus Structure analyses of the European-scale dataset (with *a priori* locations) at HGB6 (at left) and HGC111 (at right).

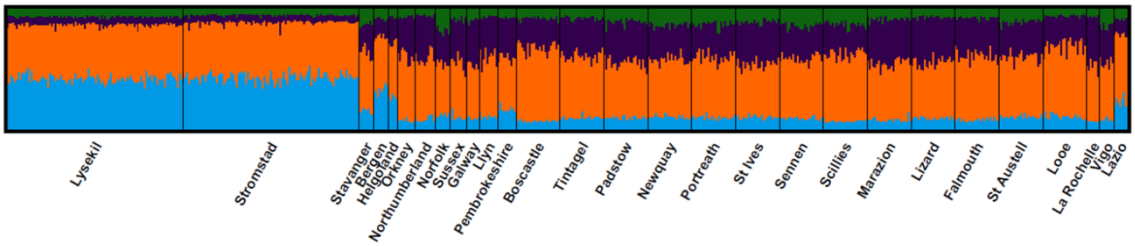

Fig. S5. Plot of assignments with four clusters. Distruct plot of convergence of K = 4 for five iterations of the full dataset from STRUCTURE models with *a priori* location data.

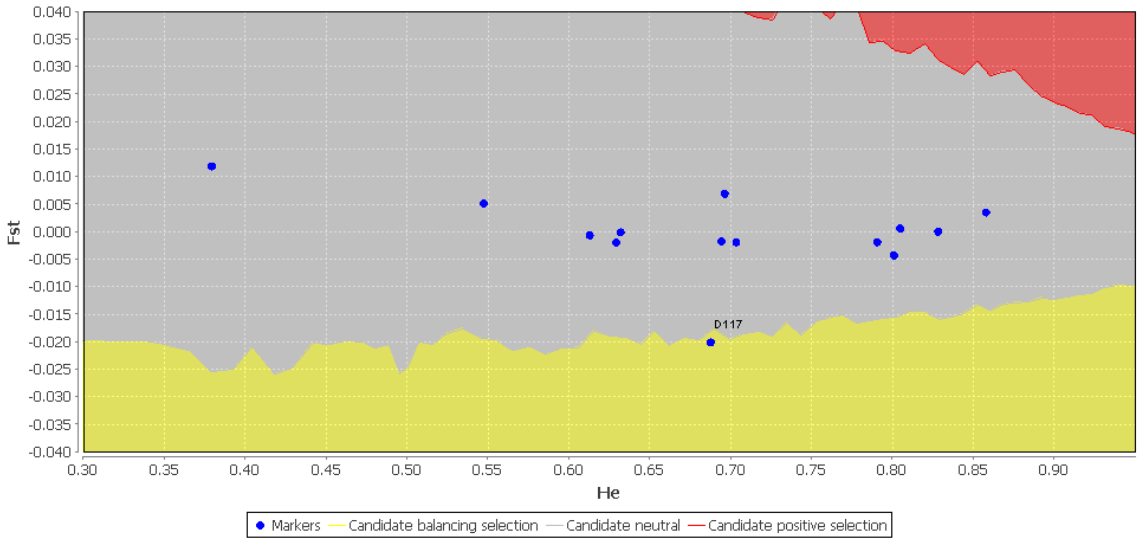

Fig. S6. Plot of markers under selection at Swedish samples only. LOSITAN plot of H_E vs F_{ST} for Swedish samples only across all markers. The grey zone denotes selective neutrality; markers (blue dots) which fall into estimated regions of directional (red) and balancing (yellow) selection are labelled.

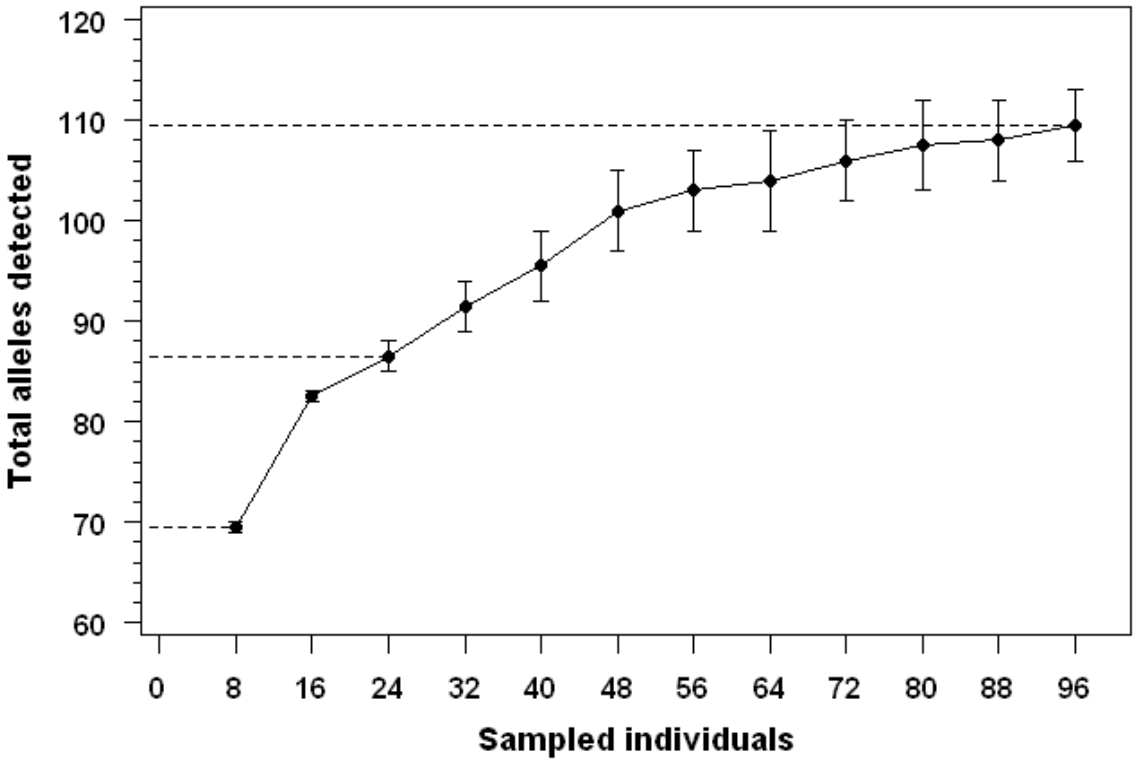

Fig. S7. Allelic discovery with number of sampled individuals. The mean total number of alleles detected across all loci in the two samples from Sweden (Lysekil and Strömstad), when reducing the sample sizes via the removal of individuals. Dotted lines show the level of detection for 96 individuals (the sizes of both Swedish samples), 24 individuals (the sizes of all fine-scale samples from Cornwall) and 8 individuals (the mean size of broad-scale samples discounting those from Sweden).