Supplementary Information

The distribution of haemoglobin C and its prevalence in newborns in Africa

Frédéric B. Piel[1,2*], Rosalind E. Howes[1], Anand P. Patil[1], Oscar A. Nyangiri[3], Peter W. Gething[1], Samir Bhatt[1], Thomas N. Williams[3,4], David J. Weatherall[5] & Simon I. Hay[1]

1 Spatial Ecology and Epidemiology Group, Tinbergen Building, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom.
2 Evolutionary Ecology of Infectious Disease Group, Tinbergen Building, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom.
3 Kenya Medical Research Institute/Wellcome Trust Programme, Centre for Geographic Medicine Research-Coast, PO Box 230, Kilifi District Hospital, Kilifi, Kenya.
4 Nuffield Department of Clinical Medicine, University of Oxford, Oxford OX3 7LJ, United Kingdom.
5 Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, United Kingdom.

This Supplementary Information includes:

Supplementary Figures:

Supplementary Tables:

Supplementary Methods:

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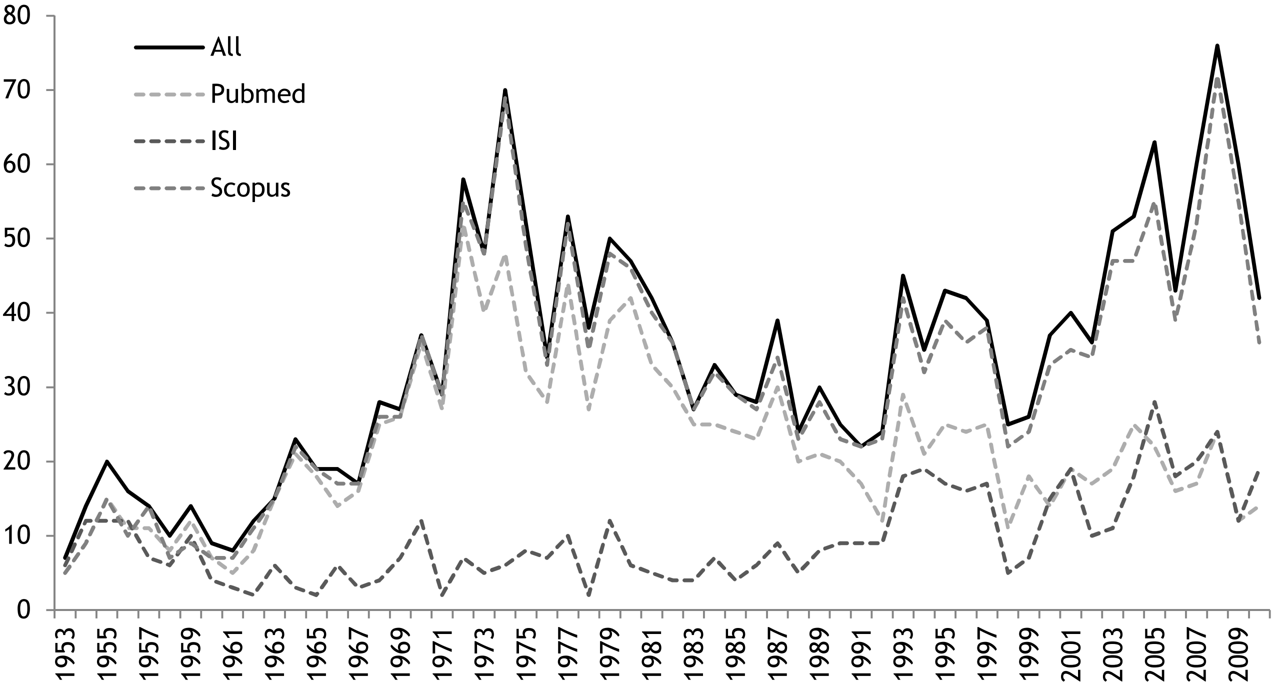
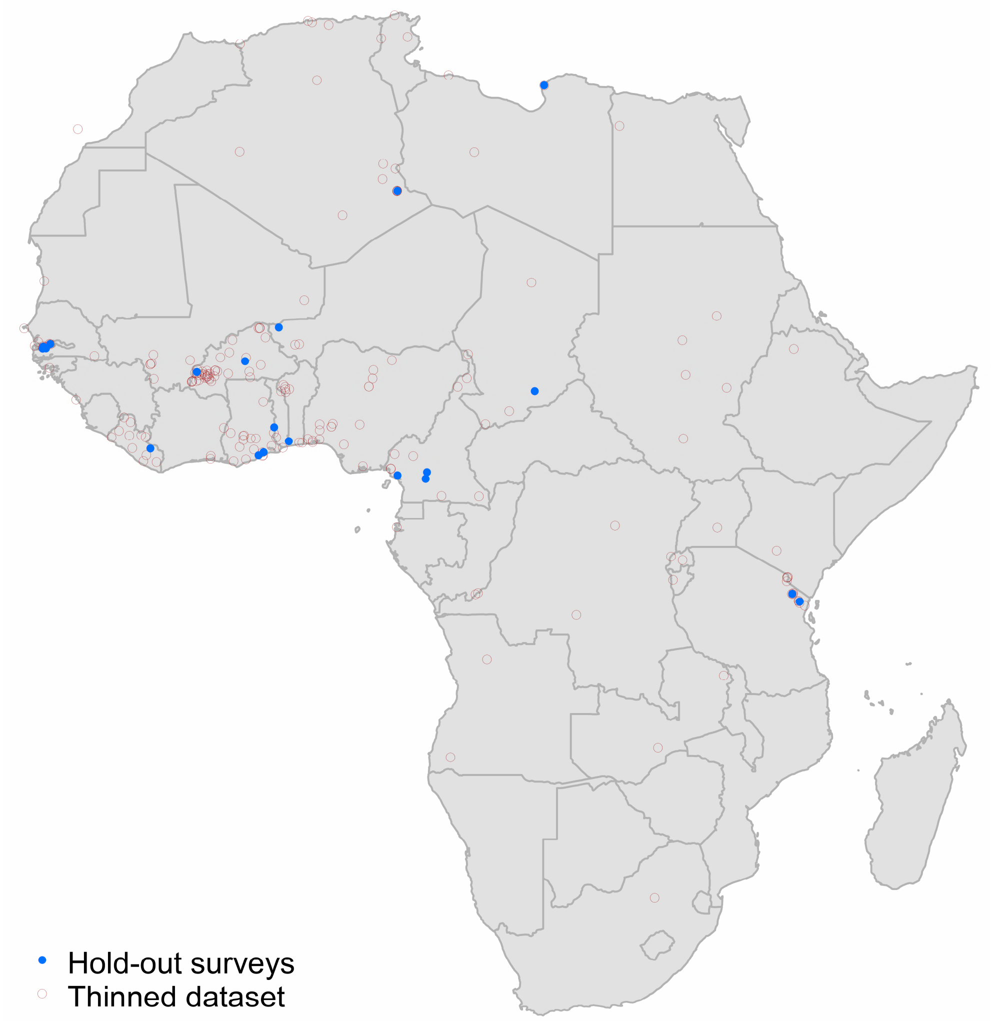
Supplementary Figures

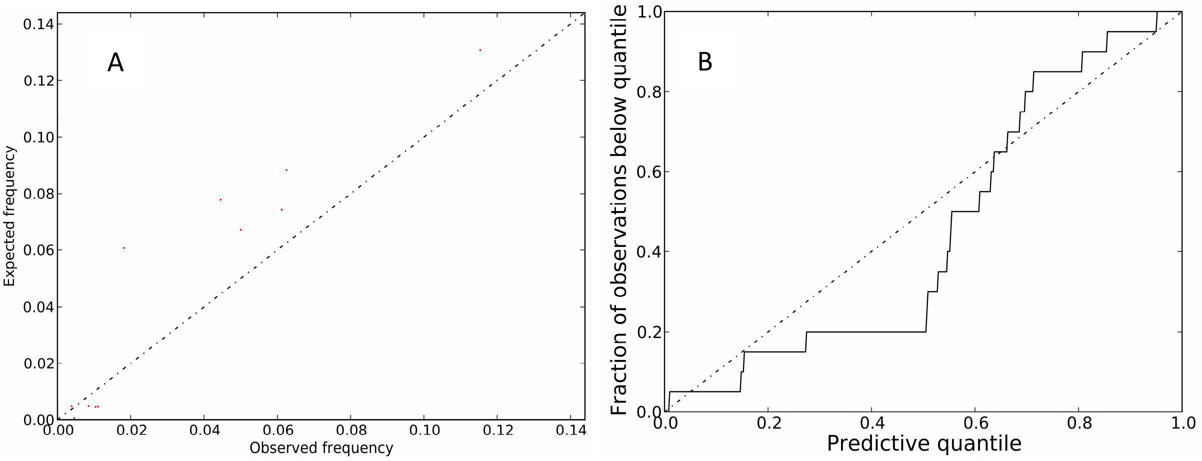
Supplementary Figure S1. Temporal distribution of the published literature on HbC. PubMed: n=1,273; ISI: n= 554 and Scopus: n=1,820. All combined after duplicate removal: n=1,963.



Supplementary Figure S2. Map of the distribution of the randomly selected subsample of the HbC data points used for the model validation, in Africa. Blue dots: thinned subset (90%); red circles: holdout subset (10%).



Supplementary Figure S3. Validation plots comparing the HbC prediction with the observed allele frequency for the data points from the hold-out subset of the data (n=20). A. Scatter plot of the observed vs. predicted allele frequency; B. Plot of the observed vs. predicted quantiles.



Supplementary Table S1: National areal prediction summaries and Monte Carlo standard errors (SE) for AC and CC newborn estimates within the AFRO WHO region

	HbC heterozygote newborns (AC)								HbC homozygote newborns (CC)							
COUNTRY	**Mean**	***SE***	**Median**	***SE***	**Q25%**	***SE***	**Q75%**	***SE***	**Mean**	***SE***	**Median**	***SE***	**Q25%**	***SE***	**Q75%**	***SE***
Algeria	3,837	*187*	3,439	*217*	1,839	*179*	7,119	*101*	62	*3*	46	*4*	15	*1*	159	*12*
Angola	2,770	*139*	1,510	*210*	257	*79*	9,539	*208*	23	*2*	4	*0*	0	*0*	102	*10*
Benin	40,503	*945*	41,915	*1,031*	32,952	*1,145*	52,400	*504*	2,004	*15*	1,892	*21*	1,188	*47*	3,200	*166*
Botswana	3	*1*	0	*0*	0	*0*	11	*4*	0	*0*	0	*0*	0	*0*	0	*0*
Burkina Faso	133,533	*1,648*	131,454	*1,499*	117,825	*156*	146,173	*3,689*	9,830	*760*	9,592	*673*	7,258	*234*	13,259	*1,613*
Burundi	192	*54*	132	*48*	31	*12*	578	*120*	0	*0*	0	*0*	0	*0*	1	*0*
Cameroon	481	*116*	400	*113*	146	*53*	1,127	*189*	1	*0*	0	*0*	0	*0*	3	*0*
Cape Verde	3	*1*	1	*0*	0	*0*	10	*3*	0	*0*	0	*0*	0	*0*	0	*0*
Central African Republic	53	*14*	27	*10*	4	*2*	187	*39*	0	*0*	0	*0*	0	*0*	0	*0*
Chad	1,620	*95*	1,282	*115*	390	*78*	4,183	*89*	16	*1*	7	*0*	1	*0*	58	*5*
Comoros	1	*0*	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*	0	*0*
Congo	267	*28*	112	*33*	21	*9*	898	*25*	2	*0*	0	*0*	0	*0*	6	*1*
Côte d'Ivoire	39,830	*1,449*	42,277	*1,584*	27,050	*929*	64,339	*1,566*	1,434	*41*	1,244	*68*	576	*45*	2,922	*67*
Democratic Republic of the Congo	2,354	*424*	1,813	*436*	594	*208*	6,213	*584*	8	*0*	2	*0*	0	*0*	30	*2*
Djibouti	5	*1*	0	*0*	0	*0*	18	*6*	0	*0*	0	*0*	0	*0*	0	*0*
Egypt	2,817	*207*	578	*200*	29	*12*	12,441	*632*	28	*3*	0	*0*	0	*0*	64	*7*
Equatorial Guinea	59	*7*	38	*8*	10	*3*	177	*9*	0	*0*	0	*0*	0	*0*	1	*0*
Eritrea	99	*18*	20	*8*	1	*0*	379	*73*	0	*0*	0	*0*	0	*0*	1	*0*
Ethiopia	1,647	*242*	470	*173*	36	*14*	6,754	*812*	10	*1*	0	*0*	0	*0*	21	*1*
Gabon	361	*6*	299	*12*	81	*16*	838	*27*	3	*0*	1	*0*	0	*0*	9	*1*
Ghana	98,589	*2,238*	98,153	*2,309*	87,225	*2,844*	110,939	*1,366*	4,843	*29*	4,707	*71*	3,601	*157*	6,546	*239*
Guinea	11,459	*397*	11,186	*385*	5,931	*73*	19,970	*842*	206	*16*	162	*15*	49	*4*	497	*34*
Guinea-Bissau	331	*23*	303	*29*	108	*24*	815	*9*	2	*0*	1	*0*	0	*0*	6	*0*
Kenya	2,095	*301*	1,048	*308*	170	*68*	7,501	*548*	8	*0*	1	*0*	0	*0*	29	*2*
Lesotho	1	*0*	0	*0*	0	*0*	2	*1*	0	*0*	0	*0*	0	*0*	0	*0*
Liberia	1,344	*40*	1,275	*51*	620	*65*	2,476	*29*	8	*0*	6	*0*	2	*0*	21	*2*
Libyan Arab Jamahiriya	562	*51*	480	*58*	173	*40*	1,313	*34*	3	*0*	1	*0*	0	*0*	10	*1*
Madagascar	5	*2*	0	*0*	0	*0*	11	*5*	0	*0*	0	*0*	0	*0*	0	*0*
Malawi	24	*8*	4	*2*	0	*0*	97	*37*	0	*0*	0	*0*	0	*0*	0	*0*
Mali	92,567	*715*	79,506	*1,147*	58,011	*2,173*	106,112	*2,318*	4,999	*318*	4,354	*105*	2,257	*88*	9,952	*1,472*
Mauritania	6,046	*139*	5,309	*166*	2,136	*44*	11,459	*168*	247	*15*	145	*6*	24	*2*	808	*95*
Mauritius	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*
Mayotte	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*
Morocco	3,979	*97*	1,878	*197*	202	*62*	14,815	*600*	56	*4*	7	*0*	0	*0*	240	*24*
Mozambique	17	*6*	2	*1*	0	*0*	68	*27*	0	*0*	0	*0*	0	*0*	0	*0*
Namibia	96	*8*	32	*9*	3	*1*	378	*14*	1	*0*	0	*0*	0	*0*	2	*0*

COUNTRY	**Mean**	***SE***	**Median**	***SE***	**Q25%**	***SE***	**Q75%**	***SE***	**Mean**	***SE***	**Median**	***SE***	**Q25%**	***SE***	**Q75%**	***SE***
Niger	41,833	*1,456*	40,670	*1,567*	24,006	*779*	69,159	*1,946*	1,425	*57*	1,196	*77*	527	*44*	3,068	*43*
Nigeria	151,207	*4,645*	148,423	*4,797*	112,961	*3,061*	197,818	*6,137*	3,407	*227*	3,099	*258*	1,822	*172*	5,948	*188*
Réunion	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*
Rwanda	616	*135*	446	*135*	129	*52*	1,554	*209*	1	*0*	0	*0*	0	*0*	3	*0*
Sao Tome and Principe	80	*1*	36	*3*	3	*1*	305	*13*	1	*0*	0	*0*	0	*0*	6	*1*
Senegal	7,639	*95*	7,326	*51*	3,508	*196*	14,548	*525*	81	*6*	56	*4*	13	*0*	230	*20*
Sierra Leone	5,247	*140*	4,508	*102*	1,575	*97*	11,076	*531*	70	*6*	40	*3*	6	*0*	228	*21*
Somalia	63	*13*	11	*5*	0	*0*	244	*67*	0	*0*	0	*0*	0	*0*	0	*0*
South Africa	15	*5*	1	*0*	0	*0*	61	*24*	0	*0*	0	*0*	0	*0*	0	*0*
Sudan	2,818	*319*	2,074	*368*	543	*179*	7,433	*310*	16	*1*	4	*0*	0	*0*	62	*5*
Swaziland	0	*0*	0	*0*	0	*0*	1	*0*	0	*0*	0	*0*	0	*0*	0	*0*
United Republic of Tanzania	951	*186*	558	*177*	123	*49*	3,033	*361*	2	*0*	0	*0*	0	*0*	8	*0*
Gambia	642	*19*	609	*24*	302	*34*	1,138	*18*	3	*0*	2	*0*	1	*0*	9	*1*
Togo	25,936	*325*	29,093	*366*	23,448	*613*	35,050	*156*	1,685	*71*	1,594	*41*	989	*31*	2,702	*282*
Tunisia	487	*64*	390	*72*	136	*42*	1,140	*59*	2	*0*	1	*0*	0	*0*	6	*0*
Uganda	4,097	*458*	2,721	*549*	618	*221*	12,531	*364*	17	*1*	4	*1*	0	*0*	69	*4*
Western Sahara	151	*1*	84	*3*	13	*3*	376	*18*	2	*0*	1	*0*	0	*0*	10	*1*
Zambia	57	*17*	23	*9*	1	*0*	221	*63*	0	*0*	0	*0*	0	*0*	0	*0*
Zimbabwe	10	*4*	2	*1*	0	*0*	40	*16*	0	*0*	0	*0*	0	*0*	0	*0*
AFRO region	**675,185**	***7,336***	**672,117**	***7,290***	**642,116**	***7,535***	**705,163**	***7,656***	**29,385**	***889***	**28,703**	***717***	**26,027**	***382***	**31,958**	***1,224***

Supplementary Table S2. MCMC output parameter values. Summary statistics presented are the mean and median values, standard deviation (std), interquartile range (IQR) and 95% Bayesian credible interval (95 BCI). The scale parameter is measured in units of earth radii. Values are presented to two significant figures.

Parameter	Symbol	Mean	Median	Std	IQR	95 BCI
Nugget variance	V	0.114	0.109	0.044	0.047	0.146
Amplitude (or partial sill)	ϕ	2.574	2.541	0.322	0.347	1.210
Scale (or range)	θ	0.490	0.493	0.010	0.012	0.037

Supplementary Methods

Inclusion criteria

Only population samples representative of the local communities were included. Detailed data given for all the ethnic groups found in the area (based on the information provided in the original source) were recorded as separate entries but entered as a single population survey in the model. Surveys targeting only specific ethnic groups (e.g. African Americans in North America) were excluded as in most cases the proportion of that particular ethnic group in the general population was unknown, as well as the allele frequency in the other ethnic groups present at the sampling site. Surveys focusing on hospital patients (e.g. minor ailments, fever or malaria) were also excluded, as they may represent biased estimates of the frequency in the general population.

When possible, authors were contacted for additional information concerning their studies in order to obtain missing information necessary to assess the quality of their results. Because of the possible misidentification of HbC and HbE with commonly used electrophoretic methods[1], only studies in which the identification of HbC appeared unambiguous were included.

No constraints were placed on sample size as, in a model-based geostatistical (MBG) framework (see below), there is no rational for excluding small sample sizes. This is because, on the one hand the model weighs the information content of each survey in accordance with a binomial sampling model, therefore down-weighting the information from very small samples, and on the other hand the uncertainty in relation to the sample size is explicitly modelled by this technique[2]. Nevertheless, case reports were excluded from this study as they did not match our criterion for representativeness of the local communities.

The number of normal (A or *neg*) and abnormal (C or *pos*) alleles observed was used as input for the model. For example, in a sample of N individuals tested in which n_{AA}, n_{AC}, n_{CC} were found to be AA, AC and CC individuals respectively, HbA and HbC allele frequencies are:

$$p = \frac{(2 * n_{AA}) + n_{AC}}{2N}$$

$$q = \frac{(2 * n_{CC}) + n_{AC}}{2N}$$

We assumed all populations to be at Hardy-Weinberg equilibrium (HWE)[3,4]. Although assuming random mating is inaccurate in most communities in northern Africa due to high consanguinity levels[5], , only scarce data on this factor was available in the data sources used for the present study and there is currently no consistent database allowing to quantify these factors regionally or globally.

Georeferencing

The geographic location of each survey was determined as precisely as possible using a georeferencing protocol adapted from Guerra *et al.*[6]. Author descriptions of survey sites were used to locate the sampling sites. Geographic coordinates (in decimal degrees, WGS84) were identified in various global gazetteers including the Encarta Reference Library 2007 (Microsoft Corporation, Redmond, WA, USA), Geonames (National Geospatial-Intelligence Agency. http://geonames.nga.mil/ggmagaz/) and Global Gazetteer Version 2.2 (Falling Rain Genomics Inc. http://www.fallingrain.com/world/index.html). Surveys were categorised according to the area that they represented: points (≤10km^2), wide areas (>10 and ≤25km^2), and small (>25 and ≤100km^2) or large polygons (>100km^2). Polygons were digitised and centroids calculated in GIS software (ArcView 3.2 and ArcMap 10.0, ESRI Inc., Redlands, CA, USA). A similar method was applied to surveys which could only be georeferenced to the district (admin2 unit) level. Surveys reported only to province (admin1 unit) or country (admin0 unit) level were considered to lack sufficient geographical specificity and were thus excluded. The geographic coordinates (latitude and longitude) were used as input in the model.

Bayesian model-based geostatistical framework

a. Model

In this section, we describe our Bayesian spatial model for the HbC allele frequency surface $(.)$ in Africa. *C* takes as its argument an arbitrary location on the Earth's surface within this continent. The posterior $[C]$ induces a posterior $[CC]$ for the HbC disease frequency surface $CC(.)$ since, using the Hardy-Weinberg[3,4] assumption that an individual's two copies of each allele are chosen independently from a gene pool, $CC(x) = C(x)^2$. We computed summaries of $[CC(x)]$, such as the mean $E(CC(x))$ and the variance $Var(CC(x))$, at each location x, to produce the maps related to haemoglobin C disease frequency in newborns.

The model differs from the model employed by Piel *et al.*[7] because, while conducting the analysis for the current paper, we diagnosed a lack of fit in our previous model that did not have a substantial effect on the main summaries of interest of the posterior for the allele frequency, but did cause serious errors in the posterior for homozygotes $[CC]$. This point is discussed further below.

The national-level disease burden $D(A)$ in nation A can be computed from CC, the birth rate b_A in *A* and the population density surface *N* using the areal integral:

$$D(A) = \int_A b_A N(x) CC(x) da$$

This is a deterministic transformation of CC and therefore of C, so theoretically the posterior $[C]$ induces the posterior $[D(A)]$. However, for reasons discussed by Patil *et al.*[8], it is prohibitively expensive to sample from this posterior. We produce approximate samples using a method described below.

b. Prior

We model C as a non-linear transformation of a Gaussian random field[8] $f(.)$, plus a random field $\varepsilon(.)$ that associates an independent normally distributed value with each location on the earth's surface. Specifically,

$$C(x) = g\big(f(x) + \epsilon(x)\big)$$

The link function *g* maps the random variable $f(x) + \epsilon(x)$, which can be any real number, to the interval (0,1), so $C(x)$ can be used as a probability or prevalence. We used a non-standard link function, which is described below.

The prior for *f* is parameterized so that the constant mean function $M(x) = m$, and the standard exponential covariance function $Cov(x, y) = \phi^2 \exp\frac{|x-y|}{\theta}$ with amplitude parameter *φ* and range parameter θ, with suitable priors assigned to the scalar parameters *m, φ* and *θ*:

$$p(m) \propto 1$$

$$\phi \sim Exponential(.1)$$

$$\theta \sim Exponential(.1)$$

$$f \sim GP(M, Cov)$$

The units of x, y and θ are earth radii, and m and φ are unitless. The unstructured component $\varepsilon(x)$ is modeled as normally distributed with unknown variance V:

$$V \sim Exponential(.1)$$

$$\epsilon(x) \overset{iid}{\sim} Normal(0, V, \alpha)$$

A regional approach was privileged over a global analysis due to the paucity of datapoints outside the African continent.

c. Likelihood

Adopting the Hardy-Weinberg assumption[3,4], if n_i individuals are sampled at the *i*'th observation location o_i (for a total of $2n_i$ chromosomes), the probability distribution for the number k_i of copies of the HbC allele that will be found is binomial, with probability $C(o_i)$:

$$k_i \sim Binomial(2n_i, C(o_i))$$

d. Flexible link function and empirical Bayesian analysis

The link function g for binomial data is usually taken to be the inverse logit function:

$$g(x) = logit^{-1}(x) = \frac{\exp x}{1 + \exp x}$$

Piel *et al.*[7] employed this model. Applying the change of variables formula, the induced prior for *C(x)* is:

$$p\big(C(x)\big) = \frac{1}{C(x)\big(1 - C(x)\big)} Normal\big(logit\big(C(x)\big); m, \phi^2 + V\big)$$

Note that this is essentially a two-parameter family of probability distributions, since φ and V appear only in the sum.

When we initially attempted to fit Piel et al.'s model to the current dataset and predict CC, we found that, when the local distribution above is fitted in areas where datapoints are highly clustered, the best fitting values of m and $\phi^2 + V$ result in implausibly long right-hand tails for the predictive distribution of prevalence in the next observation at x. Although the standard summary statistics, including the upper 95% credible interval, were consistent with the local dataset, strikingly high allele frequencies $C(x)$ (greater than 30%) were predicted with small but practically significant probability (0.1% or so).

This particular type of lack of fit was not a major issue for predictions of $C(x)$ because the bulk of the predictive distribution was roughly consistent with the dataset, but the long right-hand tail translated to an even longer right-hand tail for $CC(x)$, which contained enough mass to skew all of the standard summary statistics. For example, the predicted mean of $CC(x)$ in some areas exceeded 5%, which is highest than all but the observed values in the dataset.

To remedy this problem, we attempted several strategies including employing Stukel's link function[9] in place of the more standard inverse logit, and modelling $f(x) + \epsilon(x)$ as a skew-Gaussian process[10] rather than the standard Gaussian. The skew-Gaussian approach showed indications that it would solve the problem, but eliminated the crucial conjugate relationship between $f(x)$ and $f(x) + \epsilon(x)$, and we were unable to devise a successful MCMC scheme.

Ultimately, we employed an alternative flexible link function:

$$h(x) = \sum_{i=0}^{3} ¢_i x^i$$

$$g = logit^{-1} \circ h$$

We were unable to infer $¢_i$ jointly with the other model parameters in a fully Bayesian manner due to poor MCMC mixing, so we adopted an empirical fitting approach inspired by data pre-processing steps employed in classical geostatistics, which improved the fitting of the model to the data. The polynomial coefficients for such function are specific to the dataset. The set of coefficients used, corresponding to an invertible function and fitting the empirical cumulative distribution function (CDF) was:

$$y = -0.072328175x^3 + \ 1.105591388x^2 + 0.048698858\text{x} + \ 0.004114882.$$

e. Empirical Bayesian approach to fitting the polynomial coefficients

For each observation, (n_i, k_i), we first obtained the posterior expectation of the gene pool-wide prevalence of HbC with uniform prior density on *[0,1]*:

$$\hat{p}_i = \frac{k_i + 1}{2n_i + k_i + 2}$$

We discarded values for which *2n$_i$* was below 50. Then, we inferred the parameters $\tilde{m}$ and $\tilde{V}$ of the non-spatial Bayesian model:

$$p(\hat{p}) = \prod_i \frac{1}{\widehat{p_i}(1-\widehat{p_i})} Normal\big(logit(\widehat{p_i}); \tilde{m}, \tilde{V}\big)$$

We then plotted the posterior predictive CDF of $logit(\hat{p})$ against its empirical CDF, and fit the coefficients *Ȼ* of the cubic polynomial function *h* to the points using least squares, subject to the constraint that *h* must be invertible (or, equivalently, monotone).

In the Bayesian analysis of the full spatial model, the fitted values of Ȼ were taken as known and fixed. Although this empirical procedure is admittedly informal, the resulting nonstandard link function did substantially improve the fit of the model to the data.

f. Prior predictive constraint

Epidemiological data on haemoglobin C (HbC) tend to be opportunistic, i.e. relatively abundant in areas where it is expected to be found (i.e. West Africa), but rare elsewhere, even within Africa. Finding a carrier in a population survey conducted areas anywhere on this continent is nevertheless plausible.

Even with the more flexible model for *ε(x)*, the predictive distribution in areas of low data coverage exhibits long right-hand tails, and the predicted mean value of *C(x)* in these regions is surprisingly high. The ideal solution to this problem would be to gather further data on the prevalence of HbC in areas where data are missing from sources such as health service reports, and incorporate it in the model. Given the obvious logistical difficulties associated with this ideal approach and the lack of spatial precision when such data is available, we found it more practical to supplement the dataset with expert opinion.

Perhaps the best way to incorporate this expert opinion would be as ‘soft data’, as described by Christakos[11] among others. However, producing defensible local pseudo-observations of HbC allele frequency all but requires the data collection process that we sought to avoid. In addition, using soft datapoints would increase the number of spatial locations at which the Gaussian random field *f* has to be imputed, increasing the computational expense of fitting the model.

As a compromise, we elected to constrain *φ*, *m*, and *V* in such a way that the prior predictive distribution of *C(x)*, before the data are incorporated, puts probability mass of 1×10^{-4} or less on values in excess of .0001. In other words, we constrained 99.99% of the prior predictive probability mass between allele frequencies of 0% and 0.01%.This constraint arguably induces a lack of fit by forcing *f(x)* to depart from its prior mean by many standard deviations in areas where HbC allele frequency is known to be high; but it does remedy the implausibly high predictive values in some parts of Africa, and does not seem to adversely affect the fit in other areas.

Multiple combinations of the threshold allele frequency and maximum probability values were tested in order to assess the performance of the model (not shown). The parameters presented here represented to best compromise in terms of i) lowering the prediction in peripheral areas for which no data was available; ii) visually checking the appearance of summary maps; iii) checking our areal estimates against existing estimates. This can be seen as an informal way of bringing national reporting data into the model without incorporating it directly; iv) checking the mean error and mean absolute error.

g. Fitting the model

The model was fitted using a Markov chain Monte Carlo algorithm[12] implemented in the programming language Python using the Bayesian analysis package PyMC[13].

The scalar parameters φ, m, θ and V were updated jointly using Haario, Saksman and Tamminen's adaptive Metropolis algorithm[14], as implemented by PyMC's AdaptiveMetropolis step method. Each value $\varepsilon(o_i)$ at observation location o_i was updated separately using the standard one-at-a-time Metropolis algorithm. The distribution of the Gaussian random field at the observation locations, $\{f(o_i)\}$, is conjugate to the distribution of $\{\epsilon(o_i) + f(o_i)\}$, so we updated $\{f(o_i)\}$ by sampling from its full conditional distribution. MCMC output parameter values are summarised in Supplementary Table S2.

h. Mapping procedure

Interpolating spatially sparse survey data to predict an allele frequency across a wide region results in predictions of which the level of certainty (or uncertainty) varies spatially as a function of the density, quality, and sample size of survey data available. Spatial heterogeneity of the frequency, known to be high in some areas for other haemoglobinopathies such as HbS[7,15], also influences this uncertainty. A Bayesian MBG framework[16] generates a posterior predictive distribution rather than a unique value, therefore allowing estimation of the uncertainty of the prediction for each pixel. In addition to the posterior predictive distributions of HbC allele frequency, HbAC and HbCC genotype frequencies were also generated directly by the model. Because these are non-linear functions of the allele frequency, it would be incorrect to produce summary maps of these quantities from those of allele frequency using GIS software[8].

The uncertainty is a crucial measure of the accuracy of the prediction. From the complete range of possible uncertainty intervals available from the model's output, we chose here to use the inter-quartile range (IQR) of the posterior distribution[17], corresponding to a 50% probability. This corresponds to the *mbg-map* command of the generic MBG package.

i. Mean vs. median

The output of the model is a full posterior predictive distribution (PPD). A multitude of summary statistics is therefore available.[8] The most common ones are the mean, the mode and the median.

The main advantage of the mean is that it can be used correctly to predict means of other quantities using GIS software, because the mean of a sum is equal to the sum of the means. The mean could therefore be used to compare the regional areal prediction with the sum of the national areal predictions. Because the estimates at national and regional scales were calculated independently, we did not expect to obtain equal values, but we expected them to be consistent with the Monte Carlo standard error (SE) obtained from the ten repetitions conducted at each scale. We therefore used the mean to check the sanity of our independent estimates at national and regional scales. All the sums of the mean areal estimates corresponding to sub-spatial units fell within the SE range of spatial units areal estimates.

The main advantage of the median is that it can be used in combination with the interquartile range to give a better picture of the overall prediction, particularly when the PPD is highly skewed, and its associated uncertainty. Because the sum of the median is not equivalent to the median of the sums, important differences can be observed between the regional estimate (AFRO) and the sum of national estimates. Although counter-intuitive, this is statistically correct in the present context.

j. Model validation

In addition to the model-based representations of prediction uncertainty provided by the MBG framework, the model's predictive ability was quantified by assessing the disparity between the prediction and the observed allele frequency using a validation subset of the data. Ten percent of the data (n=20), randomly selected, were held out from the dataset. The model was run in full using the thinned data set (n=186) to generate HbC PPD for comparison with known values at the locations of the held-out data (Supplementary Figure S3a). The prediction's mean error and mean absolute error were used to assess the model's overall bias and overall accuracy respectively. The mean error is the average distance between the actual data points and the predicted values. The absolute mean error is a measure of the average magnitude of the errors in the predicted values. A procedure was also implemented to test the extent to which predicted posterior distributions at each location provided a suitable measure of uncertainty. Working through 100 progressively narrower credible intervals (CIs), from the 99% CI to the 1% CI, each was tested by computing the actual proportion of held-out prevalence observations that fell within the predicted CI. In a perfect model, 95% of true values should fall within the 95% CI predicted at each location, 50% within the 50% CI, and so on. Plotting these actual proportions against each predicted CI level allows the overall fidelity of the posterior probability distributions predicted at the held-out data locations to be assessed (Supplementary Figure S3b). This corresponds to the *mbg-validate* command of the generic MBG package.

k. Demographic data

Population density is highly variable between pixels within one country. National estimates of HbC newborns therefore depend on whether areas of high or low frequencies are highly populated or not. Rather than using crudely averaged data for each country, the use of our contemporary allele frequency map for Africa combined with high resolution population data allows us to deal with this issue. Population density data have been described in detail in Balk *et al*.[18] and calculations to adjust them to 2010 populations explained in Gething *et al*.[19]

We focus here on newborns using Hardy-Weinberg assumptions[3,4]. Assuming random mating and large population sizes, it is possible to estimate the HbC allele frequency and the proportions of each genotype (AA, AC and CC) from the number of heterozygote individuals observed in the population sample[20]. Conceptually, the number of AC and CC babies born per year can be obtained by multiplying a function of HbC allele frequency ($2p(1-p)$ and p^2 respectively) by the population living within the area of interest and the crude birth rate (CBR). Crude birth rates are not consistently available across Africa at a finer resolution than the country level, hence the use of data from the United Nations Population Prospects for the 2010-2015 period[21].

l. Areal predictions

As described previously[8], one needs to be careful when predicting integrals over spatial areas. Using traditional GIS methods, a researcher having access to a map of HbC allele frequency ($C(x)$) and desiring a national proportion of individuals with the CC genotype ($CC(x)$), would take the square of $C(x)$ and then average the values over the various pixels falling within the country of interest, weighted by population. This approach has limitations when the map of allele frequency is uncertain. Squaring the mean map for $C(x)$ does not yield the mean map for $CC(x)$ and it is impossible to produce any assessment of the uncertainty of the areal average from summary maps alone[8].

To develop a correct procedure for producing predictive distributions for national proportions, we begin by considering what we would do if we had the true map of HbC allele frequency in hand. As stated above, the national-level disease burden $D(A)$ in nation A can be computed from C, the birth rate bA in A and the population density surface N using the areal integral:

$$D(A) = \int_A b_A N(x) C(x)^2 da \qquad (1)$$

In reality, the allele frequency map C is unknown. We do not know its exact value, but we have a posterior distribution $[C]$ for it, from which samples can be drawn. Because applying $equation\ 1$ to a sample from $[C]$ generates a sample from $[D(A)]$, many samples from $[C]$ can be used to build up a histogram approximating $[D(A)]$. Summaries such as the mean, median, variance and credible intervals can be approximated using these samples.

Although it is mathematically correct, this procedure is impractical to implement. We have an approximation of $[C]$ in the form of the MCMC trace, but generating samples from it at an appropriately high resolution is extremely computationally expensive[8,22]. We use an approximate procedure based on the fact that, if z_1 is a single-element binomial process on A with intensity d,

$$\frac{\int_A C(x)^2 d(x) da}{\int_A d(x) da} = E(C(z_1)^2) \qquad (2)$$

Furthermore, if z_i is an l-element binomial process on a with intensity d,

$$E(C(z_1)^2) = \lim_{l \to \infty} \frac{1}{l} \sum_{i=1}^{l} g(C(z_i)^2) \qquad (3)$$

The expectation of the term inside the limit is equal to the left-hand term, but its variance is smaller than that of $C(z_1)$.

The pseudocode for our procedure, based on this approximation, was as follows:

```
1. Generate an $l$-element binomial process on $A$, $z_l$, with intensity $N$.
2. For each value in the thinned MCMC trace for the scalar parameters and the Gaussian
   random field $f$ evaluated at the observation locations $\{o_i\}$, $\{f(o_i)\}$,
     – Draw a value for the l-element random vector $\{f(z_i)\}$ from its full conditional
       distribution.
     – Convert these values to $\{C(z_i)\}$ by applying the inverse-logit link function.
     – Square these values to obtain a sample for the value of the desired genotype
       frequencies $\{C(z_i)^2\}$.
     – Compute the arithmetic mean of this sample and store.
```

This procedure was conducted ten times using l = 5,000 and 1,000 spatial points for the regional and national areal estimates respectively; 10% of the parameter samples in the dynamic trace, selected at random; and 1,000 iterations. As this resulted in a full PPD for each areal unit of interest, various parameters could be used to summarize the predicted estimates and their uncertainty. Here, we used the median and the interquartile range (IQR).

The code used to implement this analysis is freely available at http://github.com/malaria-atlasproject/ibd-world and http://github.com/malaria-atlas-project/generic-mbg.

m. Monte Carlo standard errors

To estimate the Monte Carlo standard error[12,16,23] attributable to the use of a set of l spatial locations rather than a high-resolution raster grid, we repeated all computations ten times and recorded the sample standard deviations of all summaries (mean, median, etc.). The point estimates that we report were obtained by aggregating the samples from all repetitions. The Monte Carlo standard errors for the national and regional areal estimates are summarised in Supplementary Table S1.

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