Population dynamics of B cell subsets – analysis and predictions

We aimed to quantify the dynamics of various subsets within mature B cell population and to understand the rules of replacement of old cells by that of new ones within each subset. We assumed that FM cells circulate freely in the lymphatic system, and so we pooled the numbers of these cells recovered from spleen and lymph nodes when modelling their dynamics. We explored the possibilities that the T1, T2 transitional subsets, or their sum, may serve as the source populations for FM cells. In order to compare the time-varying chimerism in FM cells across animals with different levels of bone marrow chimerism and different sources, we normalised the FM chimerism to that in the common progenitor population, *i.e.*T1 cells. Here, we assess the suitability of T1, T2 and pooled (T1+T2) compartments as putative source populations for FM cells and explore various mechanisms that can explain their population dynamics in mice.

Follicular Mature B cells

Replacement kinetics are consistent with FM cells being a single, kinetically homogeneous population, with cell lifetime increasing with host age

The normalised donor fraction f_d in the FM compartment stabilises close to 1 by about 120d post-BMT, implying the near-complete replacement of the compartment within that timeframe. Complete replacement suggests that the average rates of loss across host and donor cell populations are always equal. Additionally, we measured the kinetics of their expression of Ki67, a nuclear protein expressed during cell cycle and lost with a lifetime of 3-4 days following mitosis (Gossel eLife 2017 and others - see refs in that paper). Immediately following BMT the donor FM cells are highly enriched for recently divided cells, with around 80% Ki67$^{\text{hi}}$, but this proportion falls slowly to equalise with that of host cells at around 10% after approximately 100 days.

We modelled this behaviour by assuming that cells differentiate into FM B cells at a rate proportional to size of source population, and all FM cells, whether host or donor, are lost through death or differentiation at rate δ and renewed through division at rate ρ. For generality we allowed either of these rates to vary with the age of the host. We fitted each model simultaneously to the timecourses of the total size, normalised donor chimerism and the proportions of Ki67$^{\text{hi}}$cells within host and donor compartments of the FM population (Figure 1A and B; see Methods for details). We found strongest support for T1 as the source population for FM and the model in which the loss or turnover rate δ changes with host age and the division rate ρ remains constant. Specifically, total numbers of FM B cells are given by

$$\frac{\mathrm{d}N_{\text{FM}}}{\mathrm{d}t} = \phi(t) + (\rho - \delta_0 e^{-rt})N_{\text{FM}}, \tag{1}$$

where $\phi(t)$ is the daily rate of influx from the source, which changes little with host age but whose timecourse was estimated using a (nearly flat) spline, and time is measured from age 60 days, at which time the loss rate

is δ_0. This model was superior to the simplest model with constant rates of division and turnover (ΔLOO-ic = 8) and also superior to the alternative with a time-varying division rate (ΔLOO-ic = 10). We estimate that FM B cells divide slowly, on average every 290 days, and have a mean residence time (lifetime) of 30 days in 8.5 weeks-old mice. This life expectancy doubles on an average every $\sim$7 months. We also predict that approximately 3% of FM B cells are replaced each day by newly differentiated cells from the T1 population. Parameter estimates and 95% confidence intervals are in Table 2.

We also define the net loss rate λ as the aggregate of cell division and turnover (i.e. $\delta - \rho$), which decreases with time for FM cells, as δ declines. This suggests that in old animals individual FM clones and their progeny would persist longer in follicles than in younger animals, purely due to gradual increase in their survival.

No evidence for heterogeneity within FM B cells

The decline we detect in λ with host age, therefore drives a gradual slowing of the approach to stable chimerism relative to the kinetic predicted by a simple model of constant division and turnover. An alternative explanation of this time-varying kinetic is that the FM pool comprises independent sub-populations with different but constant rates of division and turnover, each fed from the T1 source. In this scenario, less persistent populations (those with a high net loss rate λ) will be replaced most rapidly after BMT, giving an initial steep upslope in chimerism. There will then follow a slower increase as the more persistent FM subpopulations (with low λ) are replaced by donor cells relatively slowly.

We fitted a model of kinetic heterogeneity assuming two independent subpopulations, allowing their relative size and their constant loss rates (δ_1 and δ_2) and division rates (ρ_1 and ρ_2) to be free parameters. However this model received lower support than the model of FM cells as a single population with turnover slowing with host age (ΔLOO-ic = 7, Table 1). Indeed there was a very weak signature of kinetic heterogeneity; the estimated loss and division rates of the two FM subpopulations were nearly equal (δ_1 = 0.11 (0.01, 0.5), δ_2 = 0.08 (0.01, 0.48), ρ_1 = 0.02 (0.0007, 0.21), ρ_2 = 0.02 (0.0007, 0.25)).

We also found no evidence for any host-donor differences in kinetics in the form of a persistent host-derived 'incumbent' population, or any change in the net rate of loss of loss with cell, rather than host, age (Table 1). For a discussion of these models, see methods [Hogan et al. PNAS 2015 Rane et al. PLoS Biology 2018].

Summary

- Most support for model in which FM B cell residence time increases with host age
- No evidence for kinetic heterogeneity (*i.e.* multiple subpopulations with different turnover, or host incumbents)
- No evidence for changes in rates of loss or division of FM cells with cell age.

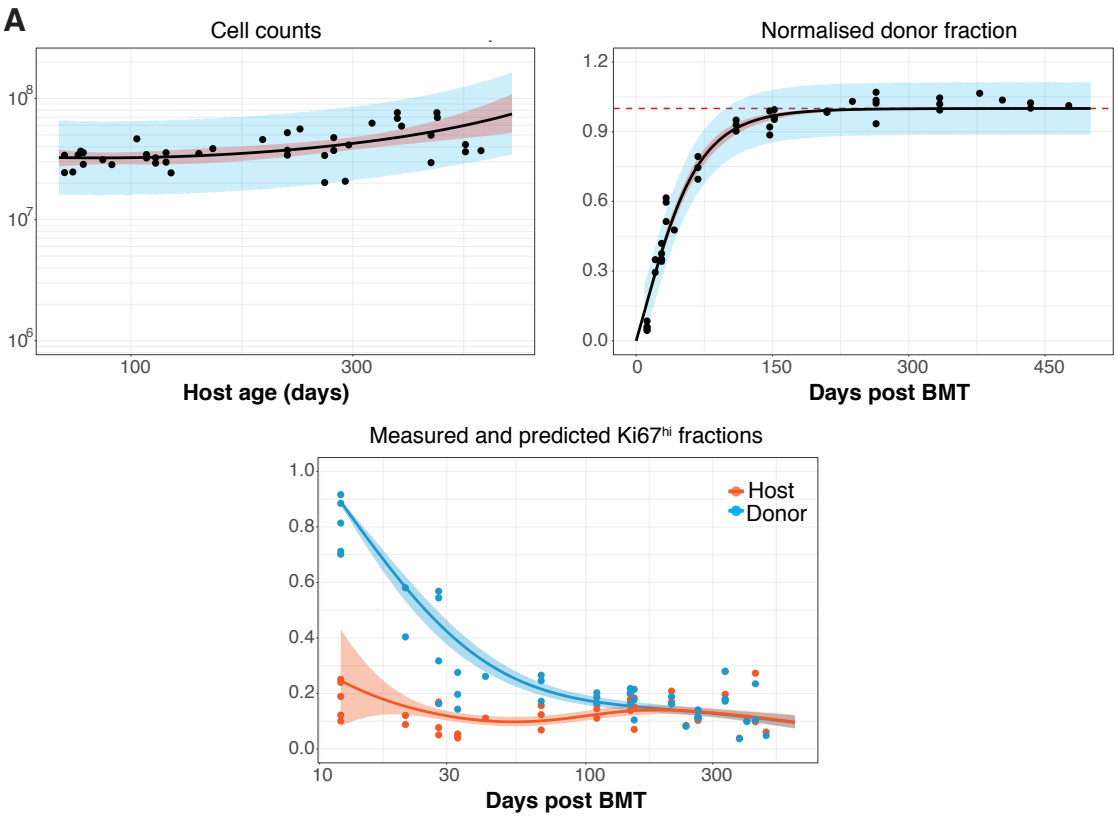

Figure 1: **Fitted and predicted population dynamics of FM B cells, using the best-fitting model in which cells divide at a constant rate and their mean residence time increases with host age.** The model was fitted simultaneously to the extended timecourses of total cell counts of FM B cells pooled from LN and spleen in busulfan chimeras, the donor fractions in FM B cells normalised to the chimerism in T1 cells and the proportion of cells that were Ki67^{hi} within host and donor FM B cells. Solid lines denote the most probable description of the observations of cell counts and normalised donor fractions and Ki67^{hi}fractions using the time-dependent model with purple envelopes indicating uncertainty in the model fit and the blue envelope indicates uncertainty in the data. Prediction intervals (4.5^{th} and 95.5^{th} percentiles) were plotted by drawing samples from the posterior distribution of parameter estimates.

	Model and ΔLoo-ic			
Source	Time-dependent	Simple homogeneous	Kinetic heterogeneity	Incumbent
T1	0	8	7	9
T2	36	43	39	42
T1 + T2	19	29	28	29

Table 1: **Comparison of models describing population dynamics of Follicular Mature (FM) B cells, pooled from LN and spleen.** Loo-ic values obtained using leave-one-out cross validation method are shown relative to that of the best fitting model, in which the rate of loss (turnover) of FM cells declines slowly with the age of the host. Predictions of more complex models were very close to those of the simple homogenous model (that is, either very little kinetic heterogeneity, close to zero incumbent cells, or effects of cells age on turnover or division rates)

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Parameter	Estimate	95% *CI
Total cell numbers at age 7 wks ($\times 10^{-6}$)	32	(24, 43)
Percent daily replacement by source at age 8.5 wks	2.7	(2.3, 3.0)
Mean residence time (days) at age 7 wks	31	(24, 39)
Mean inter-division time (days)	291	(129, 2200)
Time for mean residence time to double (days)	204	(118, 622)
Average time of loss of Ki67 expression (days)	6.0	(4.6, 7.3)

Table 2: **Parameter estimates from the best-fit (time-dependent) model for FM B cells (Spleen + LN).** *Credible intervals were estimated by taking 2.5^{th} and 97.5^{th} percentiles of the posterior probability distribution of the parameter values obtained after fitting model to the data.

Germinal Center B cells

Invasion kinetics of donor-derived GC cells differ between spleen and lymph nodes.

GC cells are known to derive directly from the mature follicular cells [REF] which circulate freely between spleen and lymph nodes [ref]. Therefore, we used pooled numbers of spleen and LN FM cells as a common source for all GC B cell subsets. We found that in both spleen and lymph nodes, the chimerism in GC compartment reaches to the level of chimerism in their source (FM) population (Figure 2 B), showing complete replacement of host GC cells by that of donor-derived cells. Surprisingly, the chimerism in splenic GC cells stabilises twice as fast than their lymph node counterparts ($\sim$120 days for spleen GCs versus $\sim$250 days for LN GCs). Due to this disparity in the invasion kinetics of donor cells between the splenic and LN GC pools, we model them separately assuming that circulating FM cells feed into both these populations with a constant rate over time.

B cells participating in GC reactions follow same kinetics of division and loss, irrespective of the age of the host.

To understand the behaviour of GC B cells we began with the simplest model, where all cells are assumed to divide and turnover with constant rates at all times, thereby implying homogeneity. This simple homogeneous model provided good visual descriptions of time-courses of cell counts and normalised donor fraction (Figure 2) with estimates of mean resident times of 0.6(0.48, 0.8) and 0.8(0.59, 1.1) days for spleen and LN GCs respectively. The simple model with constant rates of cell-division and turnover also captured the trend in $Ki67^{hi}$fractions in host and donor compartments of splenic and LN GC populations remarkably well (Figure 2). We also found that GC cells enter divisions very frequently with average times between cell cycles $\sim$0.6 and $\sim$0.8 days for spleen and LN GC cells, respectively. Majority of the GC precursors (FMs) have fewer $Ki67^{hi}$cells ($\simeq$ 10%), we speculate that almost all of the GC cells undergo multiple rounds of cell-division or are lost from the pool rapidly before losing their Ki67 expression or both.

We observed that $\sim$0.3% of LN GC and $\sim$57% of spleen GC population is replaced everyday by new cells seeding in from the follicular compartment. The estimate of substantially higher source influx into spleen GC pool is consistent with the observation of quicker stabilisation of chimerism in spleen GC cells as compared to LN GC cells. It can be inferred from this analysis that GC reactions have disparate half-lives between different secondary lymphoid organs and their dynamics are primarily regulated by tissue-specific factors.

We further tested weather varying either division or turnover with time improves on the fits provided by the simple homogeneous model. Allowing variations in ρ or δ with host age did not enhance the quality of fits and received poorer statistical support (ΔLOO-IC$_\rho$ = ? and 5 andΔLOO-IC$_\delta$ = 0 and 4.9 for splenic and LN GCs, respectively) as compared to the simple homogeneous model. Additionally, the rates of change of ρ or δ estimated by the time-dependent model were extremely small with confidence intervals spanning across zero, suggesting that the given data can be explained without any changes in division or turnover. Therefore, we speculate that the time-dependent variations in the host environment have little or no effect on the population dynamics of B cells participating in GC reactions.

GC population is maintained by continuous seeding of follicular cells into a kinetically homogeneous population over time.

The complete replacement that we see in GC cells can also be explained by the presence of kinetically heterogeneous subpopulations that divide and turnover at different but constant rates. This model of kinetic heterogeneity assumes that the GC population is comprised of fast and slow subsets (as discussed in FM B cells section). We found that the kinetic heterogeneity model failed to improve on the fits from simple homogeneous model (ΔLOO-ic = 0 and 1.1 for spleen and LN GCs respectively, Table 3) and produced strikingly similar estimates of loss rates and division rates for both fast and slow subsets in both spleen and LN GC cells.

We also tested the variant of the kinetic heterogeneity model where the host and donor cells behave differently due to the presence of a persistent incumbent subpopulation in the host compartment. We assume that this incumbent population is established during neonatal stages as the early waves of self-renewing B cells populate the peripheral compartments that are resistant to displacement by new cells. The incumbent model returned visually similar fits to the time-course of total counts and normalised donor fractions as that of the simple homogeneous model (not shown). It also failed to differentiate from the simplest model statistically despite having an extra parameter (Table 3). Moreover, the estimates of loss and division rates (δ_{INC} and ρ_{INC}) of the incumbent population were nearly equal to the loss and division rates of the displceable subset, showing very weak signature of heterogeneity. These results favour the possibility that GC populations despite being extremely dynamic are kinetically homogeneous and are sustained by constant feeding from follicular B cells.

Our analysis of FM population dynamics shows that the total size of FM compartment increases with time, due to a gradual decline in their turnover (Figure 1A, left panel). We tested whether the rate of influx of FM cells into the GC compartment also varies with host-age, so as to maintain the inflow of constant number of cells over time. We found that allowing the rate of influx to vary with host-age, results in very poor descriptions of the normalised donor fractions in both, spleen and LN GC populations (Δ LOO-ic of ?? and ?? respectively) and hence rejected this possibility. Therefore, follicular cells maturing into the GC compartment at any given time are proportional to the size of the FM population at that moment.

Summary/Discussion

- GC cell dynamics vary between spleen and lymph nodes, as observed by slower replacement kinetics of LN GC cells as compared to their splenic counterparts. Accordingly, our analysis predicts longer persistence of GC cells in lymph nodes than in spleen.
- Simple homogeneous model with constant rates of cell-division and turnover explains GC cell dynamics and predicts the kinetics of $Ki67^{hi}$fractions remarkably well, in both spleen and lymph nodes. This may suggests that under the influence of very strong antigen derived signals the mean inter-division and residence times of all cells within the GC pool, remain constant across different instances of germinal centre reactions, over time. The strong signals from antigen and T helper cells wash out the time-variability in turnover of their precursors (FMs)?
- Prolong GC reactions in response to viral antigens and gut microbes (Adachi et al. 2015, Bachman et al. 1996, Kasturi et al. 2011) may allow B cells to reach higher degree of affinity maturation $\rightarrow$ means to cope with constant antigenic drifts. An important question is whether chronic GC response consists of long-lived GC cells or constant invasion of short lived cells maintaining a long-lived steady state? Our analysis favours second scenario.

- Ki67 in GC is result of active division and turnover and is not source derived. FM population has 10% $Ki67^{hi}$cells while GC are $\sim$ 95% $Ki67^{hi}$.

	Model and ΔLoo-ic			
population	Simple homogeneous	Time-dependent	Kinetic heterogeneity	Incumbent
LN GC cells	0	4.9	1.1	2.9
Spleen GC cells	0	0	0	1

Table 3: **Comparison of Loo-ic values obtained using leave-one-out cross validation method are shown for different models fitted to cell counts and donor fractions in spleen and LN GC B cells normalised to the chimerism in FM cells (spleen + LN).**

Population	**Parameter**	Estimate	95% CI*
Lymph node GC cells	Total cell numbers at age 8.5 wks ($\times 10^{-3}$)	62	(23, 188)
	Percent daily replacement by source at age 8.5 wks	3.3	(2.8, 3.5)
	Mean resident time (days)	0.80	(0.58, 1.1)
	Mean inter-division time (days)	0.80	(0.59, 1.1)
	Average time of loss of Ki67 expression (days)	5.6	(4.2, 7.2)
Splenic GC cells	Total cell numbers at age 8.5 wks ($\times 10^{-3}$)	7.2	(1.8, 30)
	Percent daily replacement by source at age 8.5 wks	57	(38, 97)
	Mean resident time (days)	0.61	(0.49, 0.80)
	Mean inter-division time (days)	0.60	(0.48, 0.79)
	Average time of loss of Ki67 expression (days)	6.2	(4.9, 7.7)

Table 4: **Parameter estimates from the best-fit (simple homogeneous) model for spleen and LN GC B cells.** *Credible intervals were estimated by taking 2.5^{th} and 97.5^{th} percentiles of the posterior probability distribution of the parameter values obtained after fitting model to the data.

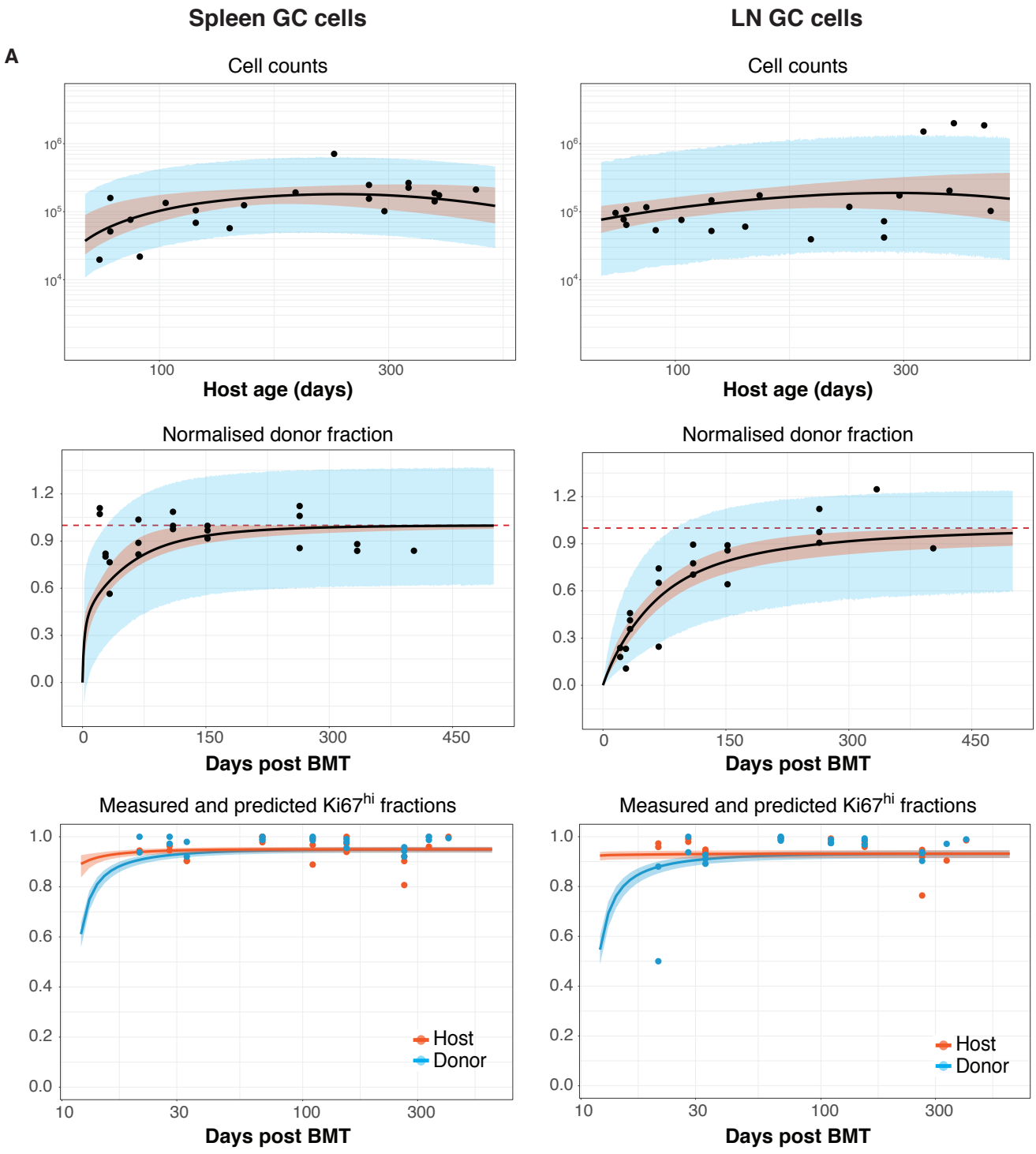

Figure 2: **Fitted and predicted population dynamics of spleen and LN GC B cells, using the best-fitting model in which cells the net loss rate of cells remain constant with time.** The model was fitted simultaneously to the extended time-courses of total cell counts of spleen and LN GC B cells pooled in busulfan chimeras, the donor fractions normalised to the chimerism in FM B cells and the proportions of Ki67^{hi}cells within their host and donor compartments. Solid lines are the fit from the simple-homogeneous model to the observations (closed circles) of cell counts, normalised donor fractions and the proportion of cells that were Ki67^{hi} within host and donor spleen and LN GC B cells, with orange envelopes indicating uncertainty in the model while blue envelope indicate uncertainty in the data. Prediction intervals (4.5^{th} and 95.5^{th} percentiles) were plotted by drawing samples from the posterior distribution of parameter estimates.