CRN tutorial: Gaussian quantitative genetic model

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General introduction

This tutorial provides a gentle introduction into the application of a covariance reaction norm (CRN) model in R for quantitative genetic analysis, focusing on the simplest case of a multivariate Gaussian model with single measures per subject. CRN analyses can be conducted using the Stan statistical programming language. Stan is an open-source programming language for estimating complex probabilistic models, which can interface with multiple statistical environments such as R. Stan facilitates fully Bayesian inference using a state-of-the-art Markov Chain Monte Carlo (MCMC) sampling technique known as the No U-Turn Sampler (NUTS), which has been found to perform particularly well for quantitative genetic analysis. Stan is thus an ideal platform for flexibly estimating CRNs.

Stan uses its own language for writing probabilistic models, including a variety of built-in functions designed to aid in efficient computation. The biggest conceptual hurdle for new users of Stan is likely to be the absence of an intuitive R-like syntax for specifying model formulas, such as formulas like `y ~ x + (1|z)` that can be used to quickly specify complex generalized linear mixed-effects models. These formulas facilitate highly efficient statistical modeling, but do so at the cost of limiting users' ability to specify atypical model structures. Instead, Stan provides the benefit of nearly unlimited flexibility in model specification, with the added cost of a steeper learning curve. In particular, models must be formally specified with mathematically appropriate likelihood functions, rather than this process being handled on the back-end through textual inputs from the user such as `family= poisson(link = "log")`. This may at first seem like a cumbersome task, but it provides a degree of independence and creativity for data analysis that is otherwise unavailable. It is this autonomy that makes it possible to unbiasedly estimate CRNs in Stan, which to the best of my knowledge cannot be accomplished with any other mainstream statistical software. Nonetheless, it is important to recognize that some practice and trial-and-error will be required to gain competency and comfortability with Stan. Therefore, I encourage those interested in CRN analysis to review the Stan Reference Manual, as well the extensive collection of Stan Case Studies, which will provide a more robust foundation for extending the basic models prevented here to more complex scenarios.

This tutorial will explain the basic structure of a Stan script in the process of introducing the CRN implementation. For the present purposes, it suffices to know that a `.stan` file for estimating a Bayesian model in Stan can be composed in any text editor (e.g. RStudio or using `write()` directly in R) and will always be composed of a series of programming blocks that are processed sequentially.

```
write("// for Stan comments
    functions{...} // Stan models are composed of
    data {...} // multiple programming blocks
    transformed data {...} //only data, parameters, and model
    parameters {...} //blocks are necessary
    transformed parameters {...} //other blocks are optional
    model {...}
    generated quantities {...} ",
  "mod.stan")
```

The `transformed data{}`, `transformed parameters{}`, and `generated quantities{}` blocks are optional and can be used to create additional quantities of interest beyond the initial data provided for the Stan model in `data{}`, the essential model parameters estimated in `parameters{}`, and the likelihood function and priors specified in `model{}`. The utility of these optional blocks will be explored further below. Importantly, any quantities specified in `model{}` will not be saved in the output of the Stan model after estimation. As will become apparent in subsequent sections of the tutorial, this feature is very helpful for saving memory in a complex analysis such as a CRN model.

In most statistical software, empirical data are input with a single matrix or dataframe. For Stan, a list is instead required with data for each scalar (real or integer), vector, or matrix object declared in the `data{}` block of the corresponding `.stan` file. For example, we can make a list `stan.dl`

```
stan.dl = list(N = nrow(df), #sample size
                 I = length(unique(df$id)), #number of subjects
                 P = ncol(X), #number of predictors
                 id = df$id, #index subjects per observation
                 X = X, #design matrix of predictors, incl. intercept
                 A = A, #relatedness matrix
                 z1 = df$z1, #response variable 1
                 z2 = df$z2 #response variable 2
                 )
```

It is essential that every quantity declared in `data{}` is present in the supplied data list, and there cannot be any NAs in the supplied list. This means that in some cases, it will be necessary to either drop cases or conduct missing data imputation manually prior to the analysis, or to input an integer that is used within the Stan model to differentiate missing values (e.g. -99 indicates NA). For general application, it also useful to supply environmental covariates as a design matrix `X` rather than as individually specified variables. This can be easily accomplished in R.

```
X = data.frame(model.matrix(response ~ pred1 + pred2 + pred3 * pred4, data = df))
```

The names of the variables in the data list `stan.dl` should be coded in `data{}` along with their expected dimensions, which ensures that inappropriate data structures or likelihood functions will throw errors. For instance, in the general case, the data block might look something like this. Note that Stan uses `//` rather than `#` for comments.

```
data {
  int<lower=1> N; //length of response vector/total observations
  int<lower=1> I; //number of individuals
  int<lower=1> P; //number of covariates

  int<lower=1> id[N]; //N length index linking observations of z to subject ID
  matrix[N,P] X; //design matrix of environmental covariates
  matrix[I,I] A; //relatedness matrix

  vector[N] z1; //vector of real valued responses (e.g. for Gaussian model)
  int z2[N]; //response variable of counts (e.g. for Poisson model)
}
```

This declarative approach requires that particular attention is given to the order of data input to the model, as values will need to be appropriately aligned and indexed throughout the model specification. This is because Stan will not take character strings for indexing subjects (e.g. “monkey1”, “subjectA015”, “juvenileB”, etc.) or other random effects such as group identity, season, time of day, etc. This can also be easily accomplished in R. For instance, if one is attempting to change the character string names in an R object `df$subj` for indexing in Stan

```r
key.id = unique(df$subj) #list all unique subject IDs
new.id = seq(1:length(key.id)) #create numeric index of equal length
df$id = new.id[match(df$subj, key.id)] #create new id for Stan
```

Users will need to manually ensure that the integers used to index subjects are also appropriately aligned with the order of any other data structures corresponding to those subjects. The `A` matrix, for instance, should be arranged so that row 1 corresponds to the values expected for subject 1 and so on.

```r
dimnames(A)[[1]] = new.id[match(dimnames(A)[[1]], key.id)]
dimnames(A)[[2]] = new.id[match(dimnames(A)[[2]], key.id)]
A = as.matrix(A[order(as.numeric(row.names(A))), order(as.numeric(colnames(A)))])
```

This may seem cumbersome at first, but it allows for many benefits unavailable in more standard statistical software, such as facilitating multivariate models with heterogeneous dimensions (differing N per response variable). See the meerkat data analysis files for a concrete example.

Once the model is written in a `.stan` file and the corresponding data list is prepared, the stan model can be compiled in C++ and estimated in R using either the rstan or cmdstanr packages. Details on the installation of these packages can be found elsewhere online (see hyperlinks). This tutorial will focus on the use of cmdstanr for model estimation, as it provides utility functions for speeding up MCMC chains that are currently unavailable in rstan. We will then use functions from rstan for exploring and summarizing the posterior estimates of the CRN model. Optional packages such as shinystan can also be used to aid in inspection of model results and diagnostics.

```r
library(shinystan)
library(cmdstanr)
library(rstan)

#directory for cmdstan installation
set_cmdstan_path("...")

#compile model
mod = cmdstan_model(stan_file = "model.stan", #set appropriate directory
                    stanc_options = list("O1")) #this speeds up estimation
```

```
#estimate model
est = mod$sample(
  data = stan.dl,
  iter_sampling = 500, #how many samples per chain after warmup
  iter_warmup = 500, #warmup for MCMC chains to 'burn in' and converge
  init = 0.01, #values for initializing chains
  chains = 4,  #how many chains to run
  parallel_chains = 4, #how many in parallel (check your machine)
  adapt_delta = 0.80, #default, >0.80 aids removing divergent transitions (essential)
  max_treedepth = 10, #default, 10+ to 12 can remove treedepth warnings
  refresh = 10) #how many iterations to update progress of MCMC chains

CRN_fit = read_stan_csv(est$output_files()) #extract results into rstan format
saveRDS(CRN_fit, "CRN_fit.RDS") #save results
launch_shinystan(CRN_fit) #GUI for investigating model fit
```

Simulate multivariate Gaussian data

For this tutorial, we are going to consider the simplest case of multivariate Gaussian traits (3 response variables) with a single measure of each trait per subject. For estimating the CRN of these traits, we'll consider the additive effects of 3 continuous environmental predictors. As will generally be the case in field or laboratory research, it will be assumed that each measured level of these continuous environments, herein referred to as *contexts*, is experienced by multiple individuals. If no contexts were shared across subjects, we could not meaningfully estimate how the environment changes among-individual covariances. However, as shown in the main text, unbiased estimates can be obtained even with small numbers of individuals per context, and information can be pooled across contexts during model estimation even in cases where some contexts are only experienced by a single individual. In this example, we will assume there are 25 unique contexts across each continuous predictor experienced by 500 subjects, such that 20 individuals are measured per context assuming balanced sampling. For example, our field study might involve phenotyping 20 individuals per plot across 25 plots, with each plots' unique combination of local temperature, rainfall, predator density, resource availability, etc. serving as a multivariate environmental context for estimating the CRN.

The helper function `sim_CRN_QG()` from the `CRN functions.R` file can be used to quickly simulate data for this scenario that is appropriately formatted for Stan. This function requires arguments `N` for the total sample size, `npred` for the number of continuous environmental predictors, `Nc` for the number of contexts measured for the continuous covariates, `ntrait` for the number of phenotypic traits, and `l_es` and `u_es` for the absolute-valued lower and upper bound of effect sizes for the CRN parameters. The simulated parameter values will be drawn from uniform distributions $U(l_{es}, u_{es})$, with signs randomly flipped to allow for both positive and negative effects.

```
#custom functions (make sure to set appropriate directory)
#the file should automatically install and load any necessary packages
#that are not found in your R library
source("CRN functions.R")

#simulate data
stan.dl = sim_CRN_QG(N = 500, Nc = 25, npred = 3, ntrait = 3, l_es = 0.3, u_es = 0.6)
```

From our Stan data list, we can see the simulated multivariate normal responses

```
head(stan.dl$z, 5)
```

```
##            z1        z2         z3
## 1   -2.960651 -1.752683  0.5585328
## 1.1 -1.903928  2.566962 -2.3298299
## 1.2  2.559329  0.232979  0.8723139
## 1.3 -2.534577  1.672239 -1.6355481
## 1.4 -1.463970  2.912229  1.0770779
```

the design matrix containing the standardized, continuous environmental measures

```
head(stan.dl$X, 5)
```

```
##   X.Intercept.         X1         X2         X3
## 1            1  1.4798931 -1.2979887  1.0242250
## 2            1  1.1623586  0.1740553  0.3820382
## 3            1  1.5399547  0.1036221  0.3400949
## 4            1 -1.0097621  2.2361192 -1.8333117
## 5            1 -0.6390358  0.1710165 -1.2846423
```

the relatedness matrix among subjects (simply a random positive-definite correlation matrix)

```
stan.dl$A[1:5,1:5]
```

```
##              [,1]        [,2]         [,3]         [,4]         [,5]
## [1,]  1.000000000 -0.05849467 -0.005953564 -0.078991955 -0.041420702
## [2,] -0.058494670  1.00000000 -0.096756841  0.052342010  0.015465036
## [3,] -0.005953564 -0.09675684  1.000000000  0.001620696 -0.033395026
## [4,] -0.078991955  0.05234201  0.001620696  1.000000000 -0.002038257
## [5,] -0.041420702  0.01546504 -0.033395026 -0.002038257  1.000000000
```

the mean, variance, and canonical partial correlation RN parameters

```
stan.dl$B_m
```

```
##            [,1]       [,2]       [,3]
## [1,] -0.5510682 -0.3304345  0.5990958
## [2,] -0.5238086  0.4861180 -0.4757771
## [3,] -0.3611816 -0.3217273 -0.3215069
## [4,] -0.5030098 -0.5635266 -0.3295473
```

```
stan.dl$B_v
```

```
##           [,1]       [,2]       [,3]
## [1,] 0.4044814 -0.4858206 -0.5622857
## [2,] 0.5840890  0.3253103 -0.5429411
## [3,] 0.3203922 -0.4347445  0.3186363
## [4,] 0.5917739  0.5965077 -0.4768660
```

```
stan.dl$B_cpc
```

```
##            [,1]       [,2]       [,3]
## [1,]  0.5901370 -0.3505891 -0.5995460
## [2,] -0.5303605 -0.3171246 -0.3809773
## [3,] -0.3678121 -0.5484954 -0.4715446
## [4,]  0.3075629 -0.4135420 -0.5911267
```

and the resulting context-specific **G** matrices

```
stan.dl$Gcov[1:5]
```

```
## [[1]]
##            [,1]       [,2]       [,3]
## [1,]  4.3021500  1.9942155 -0.3247276
## [2,]  1.9942155  3.2247498 -0.5001433
## [3,] -0.3247276 -0.5001433  0.1035413
##
## [[2]]
##             [,1]        [,2]       [,3]
## [1,]  3.91669640  0.05492583 -0.7669912
## [2,]  0.05492583  1.04553189 -0.3162549
## [3,] -0.76699122 -0.31625489  0.2671090
##
## [[3]]
##            [,1]       [,2]       [,3]
## [1,]  4.6571844 -0.3735438 -0.7807001
## [2,] -0.3735438  1.1888216 -0.2195882
## [3,] -0.7807001 -0.2195882  0.2170663
##
## [[4]]
##             [,1]        [,2]        [,3]
## [1,]  0.57477283 -0.04578692 -0.76746853
## [2,] -0.04578692  0.05613298 -0.02073951
## [3,] -0.76746853 -0.02073951  4.81968398
##
## [[5]]
##           [,1]      [,2]      [,3]
## [1,] 0.5095650 0.1455216 0.2520410
## [2,] 0.1455216 0.2155951 0.2284703
## [3,] 0.2520410 0.2284703 1.5710864
```

among other simulated values and parameters necessary as input for the `CRN_tutorial_mod.stan` file. For example, the Stan model needs a matrix `cmat` indexing which subjects are observed in which contexts (rows)

```
head(stan.dl$cmat, 5)
```

```
##      [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11] [,12] [,13] [,14]
## [1,]    1    2    3    4    5    6    7    8    9    10    11    12    13    14
## [2,]   21   22   23   24   25   26   27   28   29    30    31    32    33    34
## [3,]   41   42   43   44   45   46   47   48   49    50    51    52    53    54
## [4,]   61   62   63   64   65   66   67   68   69    70    71    72    73    74
## [5,]   81   82   83   84   85   86   87   88   89    90    91    92    93    94
##      [,15] [,16] [,17] [,18] [,19] [,20]
## [1,]    15    16    17    18    19    20
## [2,]    35    36    37    38    39    40
## [3,]    55    56    57    58    59    60
## [4,]    75    76    77    78    79    80
## [5,]    95    96    97    98    99   100
```

indices linking observations to individuals `id`, contexts `c_d`, and individuals' positions in `cmat`

```
head(stan.dl$id,5)
```

```
## [1] 1 2 3 4 5
```

```
head(stan.dl$c_id, 5)
```

```
## [1] 1 1 1 1 1
```

```
head(stan.dl$idc, 5)
```

```
## [1] 1 2 3 4 5
```

counts of the number of individuals per context `cn`, the max number number of individuals in any context `cm`, and the total number of context-specific individual values to be estimated `cnt`

```
head(stan.dl$cn,5)
```

```
## [1] 20 20 20 20 20
```

```
stan.dl$cm
```

```
## [1] 20
```

```
stan.dl$cnt
```

```
## [1] 500
```

as well as the total number of observations `N` and subjects `I` (both equivalent to `cnt` in the case of balanced sampling and non-repeated measurements), the total number of contexts `C`, the total of traits/dimensions `D`, the total number of environmental predictors `P` (including a global intercept).

```
str(stan.dl, list.len = 5)
```

```
## List of 21
##  $ N    : int 500
##  $ C    : int 25
##  $ I    : int 500
##  $ D    : num 3
##  $ P    : int 4
##   [list output truncated]
```

Inspecting the code inside this function (simply type `sim_CRN_QG` into the R console) will aid in generating these values manually and specifying the appropriate stan data list for your own analysis. See the meerkat data analysis as well for a worked example of data preparation using real measurements.

Compose CRN model for Stan

Eq. 4-7 in the main text describe useful computational tricks that can be implemented in Stan for speeding up model estimation. Those interested in understanding why and how these tricks work can see the main text for further details. However, it is not essential that one fully appreciates the mechanics of these steps to carry out a CRN analysis. It is simply important to understand that some chunks of Stan code will need to be present in every CRN analysis to take advantage of these efficiency gains. As explained further below, custom functions in R can then be used to get more easily interpretable outputs from the model for summarizing and visualizing results.

Every CRN model will need to include two custom functions `sum_square_x` and `lkj_to_chol_corr` in the `functions{}` block. These functions are taken from prior work by Dan Schrage and facilitate using the environmental covariates to predict canonical partial correlations, from which effects on the genetic correlations can subsequently be derived.

```
functions {

//functions are used fom prior work by
//Dan Schrage (https://gitlab.com/dschrage/rcovreg)

  real sum_square_x(matrix x, int i, int j) {
    int j_prime;
    real sum_x = 0;
    if(j==1) return(sum_x);

    j_prime = 1;
    while(j_prime < j) {
      sum_x += x[i,j_prime]^2;
      j_prime += 1;
    }
    return(sum_x);
  }

  matrix lkj_to_chol_corr(row_vector constrained_reals, int ntrait) {
    int z_counter;
    matrix[ntrait,ntrait] x;

    z_counter = 1;
    x[1,1] = 1;
    for(j in 2:ntrait) {
      x[1,j] = 0;
    }
    // Fill the diagonal and lower triangle of x.
    for(i in 2:ntrait) {
      for(j in 1:ntrait) {
        if(i==j) {
          x[i,j] = sqrt(1 - sum_square_x(x, i, j));
        } else if(i > j) {
          x[i,j] = constrained_reals[z_counter]*sqrt(1 - sum_square_x(x, i, j));
          z_counter += 1;
        } else {
          x[i,j] = 0;
        }
      }
    }
    return(x);
  }
}
```

As noted above, `data{}` will then include statements for all of the objects in `stan.dl` that are necessary for programming the model. The data list may also include variables of interest that are not used in the Stan model, such as the context-specific **G** matrices used for simulation (`stan.dl$Gcov`).

```
data {
  int<lower=1> N; //total number of observations
  int<lower=1> C; //total number of environmental contexts
  int<lower=1> I; //total number of subjects
  int<lower=1> D; //total number of traits/dimensions
  int<lower=0> P; //total number of environmental predictors (+ intercept)

  int<lower=0> id[N]; //index linking observations to individuals
  int<lower=0> c_id[N]; //index linking observations to contexts
  int<lower=0> idc[N]; //index linking individuals to positions in cmat

  matrix[C,P] X; //environmental predictor matrix (+ intercept)
  matrix[I,I] A; //relatedness matrix

  int<lower=1> cm; //max number of individuals observed in a context
  int cmat[C, cm]; //matrix with all individuals observed in each context (row)
  int<lower=0> cn[C]; //count of individuals observed per context
  int<lower=1> cnt; //total number of individuals across contexts

  vector[D] z[N]; //multivariate normal response variables
}
```

In `transformed data{}`, any additional quantities or data objects of interested can be generated that are simply transformations of the values in `data{}`. For a CRN analysis, it will be necessary to include the following code for taking advantage of the Cholesky decomposition and QR decomposition (see Eq. 4-7 main text).

```
transformed data{
  matrix[I, I] LA = cholesky_decompose(A);
  int ncor = (D*(D-1))/2; //unique cov/cor parameters
  // Compute, thin, and then scale QR decomposition
  matrix[C, P] Q = qr_thin_Q(X) * sqrt(C-1);
  matrix[P, P] R = qr_thin_R(X) / sqrt(C-1);
  matrix[P, P] R_inv = inverse(R);
}
```

It suffices to say that we will now use `LA` in place of the `A` matrix and the `Q` matrix in place of the `X` matrix for computational efficiency. In `parameters{}`, we now put only the basic parameter values from which all other parameters of interest can be derived through transformation. For example, we do not directly specify all of the context-specific **G** matrices, but instead only specify the CRN parameters from which those matrices will be predicted in subsequent blocks. The `q` subscript is used here to indicate that these RN parameter values are defined with respect to the `Q` matrix from the QR decomposition. The RN parameters appropriately scaled to `X` can then be generated further below.

```
parameters {
  //fixed effects
  matrix[P, D] B_mq; //RN of means
  matrix[P, D] B_vq; //RN of variances
  matrix[P, ncor] B_cpcq; //RN of canonical partial correlations

  //random effects
  matrix[cnt, D] Z_G; //all context-specific additive genetic values
  cholesky_factor_corr[D] L_E; //Cholesky corr matrix for residuals
  vector<lower=0>[D] sd_E; //residual standard deviations
}
```

As is explained in Eq. 7 of the main text, these parameters need to be further transformed to derive appropriately scaled additive genetic values using a non-centered, matrix normal parametrization. This could be accomplished in the `transformed parameters{}` block. However, since we are not interested in saving posteriors for each of the individual-level predictions (population inferences are of primary concern), we can greatly reduce the resulting file size by doing these transformations in the `model{}` block instead. Any objects declared here can be used for specifying the likelihood and priors but will not be saved with the model output.

```
model {
  //predicted values from reaction norms
  //means
  matrix[C, D] mu = Q * B_mq;

  //variances
  matrix[C, D] sd_G = sqrt(exp(Q * B_vq));

  //correlations (expressed as canonical partial correlations)
  matrix[C, ncor] cpc_G = tanh(Q * B_cpcq);

  //scale context-specific multivariate additive genetic effects
  matrix[cnt, D] mat_G;
  int pos = 1; //keep track of position 1:cnt
  for(c in 1:C){
      mat_G[pos:(pos+cn[c]-1)] =
        LA[cmat[c,1:cn[c]],cmat[c,1:cn[c]]] * Z_G[pos:(pos+cn[c]-1)] *
                diag_pre_multiply(sd_G[c],lkj_to_chol_corr(cpc_G[c], D))';
      pos = pos + cn[c];
  }

//likelihood
  for(n in 1:N){
  row_vector[3] lin_pred = mu[c_id[n]] + mat_G[idc[n]];
  z[n] ~ multi_normal_cholesky(lin_pred,
                              //Chol factorized lower-tri residual cor matrix
                              diag_pre_multiply(sd_E, L_E));
  }

  //priors
  to_vector(B_mq) ~ normal(0,1);
  to_vector(B_vq) ~ normal(0,1);
  to_vector(B_cpcq) ~ normal(0,1);
  to_vector(Z_G) ~ std_normal();
  sd_E ~ exponential(2);
  L_E ~ lkj_corr_cholesky(2);
}
```

In the first steps, the model calculates the context-specific means `mu`, variances `sd_G`, and canonical partial correlations `cpc_G` using the transformed `Q` matrix of environmental covariates and accompanying RN parameters `B_mq`, `B_vq`, and `B_cpcq`. The `lkj_to_chol_corr` function is then used to return the context-specific (`c in 1:C`), lower-triangular Cholesky factorized genetic correlation matrices, which are calculated from the context-specific canonical partial correlations `cpc_G`. This correlation matrix is then scaled by the context-specific genetic standard deviations `sd_G` using the `diag_pre_multiply` function to return context-specific, lower-triangular Cholesky factorized genetic (co)variance matrices. Finally, the matrix normal parametrization is carried out for each context by moving sequentially along the matrix of standardized breeding values `Z_G` using the count of individuals per context `cn` and scaling these values by the transposed (`'`) context-specific genetic (co)variance matrix and the corresponding individuals' genetic relatedness, using the appropriate subset of `LA` indexed from `cmat`. With the appropriate additive genetic values now specified in `mat_G`, each individuals' (`n in 1:N`) trait values can be brought together into the linear predictors `lin_pred = mu[c_id[n]] + mat_G[idc[n]]` (i.e. context-specific means + breeding values) for their measured phenotypes `z[n]`. To further speed up computation, the residual (co)variance among these phenotypes is specified using the Cholesky decomposition and accompanying built-in Stan likelihood function `multi_normal_cholesky`.

In the general case, the code generating `mu`, `sd_G`, `cpc_G`, and `mat_G` can simply be copy-pasted into any CRN model irrespective of the complexity or specifics of the analysis. Researchers may instead need to include additional terms in the linear predictors `lin_pred` (e.g. other random effects, adjusted factors, offsets, etc.), declare distinct distributions (e.g. Poisson, binomial, gamma, beta, etc.) for the response variables, or use more complex indexing to bring the appropriate context-specific values into the likelihood function (e.g. if response variables differ in length due to repeated measurements). See the meerkat data analysis scripts for an example including each of these extensions. For phenotypic analysis in the absence of genetic data on relatedness, the `LA` component of the code can simply be removed to indicate the implicit assumption of independent random effects. Also note that priors specified with `std_normal()` should not be changed, while others are at the discretion of the researcher applying the model. The priors shown above are general purpose weakly regularizing priors that I recommend for the standard case.

Finally, the `generated quantities{}` block can be used to return appropriate parameter values by undoing the Cholesky and QR decompositions used for speeding up model estimation.

```
generated quantities{
  matrix[D,D] E = L_E * L_E'; //residual correlations
  matrix[P,D] B_m; //mean RN parameters for X
  matrix[P,D] B_v; //variance RN parameters for X
  matrix[P,ncor] B_cpc; //partial correlation RN parameters for X

  for(d in 1:D){
    B_m[,d]= R_inv * B_mq[,d];
    B_v[,d]= R_inv * B_vq[,d];
    }

  for(d in 1:ncor){
    B_cpc[,d]= R_inv * B_cpcq[,d];
    }
}
```

The complete model combining these code blocks can be found in the accompanying `CRN_tutorial_mod.stan` file. Any CRN model may begin by modifying this Stan file to meet specific needs of a particular analysis.

Estimate the CRN model in Stan

Let's now compile the model and estimate it with cmdstanr, using the basic approach outlined above. After the MCMC chains finish running, posterior samples are extracted into the rstan .csv format and can be nicely visualized and checked for appropriate convergence in shinystan. If the CRN model returns warnings about reaching the max treedepth during sampling, the model can be run again with a slightly higher `max_treedepth` value (e.g. 11 or 12 instead of 10). This will make the MCMC chains take longer to finish running. Warnings about divergent transitions indicate the model should be estimated again with a slightly higher `adapt_delta` value (e.g. 0.90 or 0.95 instead of the default 0.80). Further information about checking diagnostics and the convergence of the MCMC chains can be found elsewhere online.

```r
library(shinystan)
library(cmdstanr)
library(rstan)

#directory for cmdstan installation
set_cmdstan_path("...")

#compile model
CRN_mod = cmdstan_model(stan_file = "CRN_tutorial_mod.stan",
                        stanc_options = list("O1"))

#estimate model
est = CRN_mod$sample(
    data = stan.dl,
    iter_sampling = 500,
    iter_warmup = 500,
    init = 0.01,
    chains = 4,
    parallel_chains = 4,
    adapt_delta = 0.80,
    max_treedepth = 10,
    refresh = 10)

CRN_fit = read_stan_csv(est$output_files())
saveRDS(CRN_fit, "CRN_fit.RDS")
launch_shinystan(CRN_fit)
```

Summarize results

Once the model is estimated and its diagnostics are appropriately checked to ensure reliable results, we are ready to investigate our findings. We'll again use custom functions supplied in the accompanying `CRN functions.R` file to summarize the results of the CRN analysis, as well as to generate beta coefficients on the scale of genetic correlations rather than canonical partial correlations used for the Stan model. Greater details on each function and their arguments can be found in the R file. Given that Stan uses numeric rather than character labels for indexing vectors and matrices across response variables, the custom functions take an input string of trait names to output more easily interpreted results. We'll also need to pull out the posterior estimates from our model using the rstan function `extract()`.

```
#custom functions for getting results from Stan model
source("CRN functions.R")

#name of traits in order of stan.dl$z columns (1, 2, ..., D)
#(e.g. "count_egg", "limb.length", "BD1", etc.)
traits = paste0("Z",seq(1:stan.dl$D)) #z1-z3

#get posterior MCMC samples
post = extract(CRN_fit)
```

First we summarize the RN parameters for genetic variances using the `v_beta_f()` function. This function returns a dataframe with each row corresponding to a posterior sample of the beta coefficients for each trait listed in the column `element`. Keep in mind that these effect sizes are on the log scale due to the use of the log link.

```
#get posteriors of RN parameters in long format
v_b = v_beta_f(x = stan.dl$X, traits = traits, v_b = post$B_v)
head(v_b, 3)
```

```
##   element X.Intercept.        X1         X2        X3
## 1      Z1     0.311790  0.448286  0.0471734  0.459618
## 5      Z2    -0.284094  0.260815 -0.7267450 -0.141040
## 9      Z3     0.225208 -1.030270 -0.0195771 -0.724193
```

We'll summarize each effect by its posterior median (a more robust point estimate than the mean), the posterior probability in support of a positive or negative effect (in the direction of the median), and its 90% Bayesian credible interval (CI).

```
#posterior median
aggregate(.~ element, data = v_b, FUN = function(x)
  round(median(x),2))
```

```
##   element X.Intercept.    X1    X2    X3
## 1      Z1        -0.32  0.76  0.35  0.73
## 2      Z2        -0.41  0.22 -0.62  0.64
## 3      Z3        -0.43 -0.65  0.24 -0.83
```

```
#posterior probability in direction of posterior median
aggregate(.~ element, data = v_b, FUN = function(x)
  sum(sign(median(x))==sign(x))/length(x))
```

```
##   element X.Intercept.     X1     X2     X3
## 1      Z1       0.7740 0.9995 0.9155 0.9990
## 2      Z2       0.8425 0.7305 0.9825 0.9945
## 3      Z3       0.9555 0.9970 0.8970 1.0000
```

```
#posterior 90% CIs
aggregate(.~ element, data = v_b, FUN = function(x)
  round(quantile(x, c(0.05,0.95)),2), simplify = F)
```

```
##   element X.Intercept.            X1            X2            X3
## 1      Z1  -1.22, 0.34   0.32, 1.32  -0.07, 0.80   0.29, 1.24
## 2      Z2  -1.23, 0.23  -0.39, 0.81 -1.27, -0.13   0.23, 1.09
## 3      Z3 -0.99, -0.01 -1.07, -0.26  -0.06, 0.57 -1.28, -0.40
```

Results indicate that the first continuous covariate x_1 on average increased the genetic variance of z_1 ($\beta_{\sigma^2_a} = 0.76, p_+ = 0.99$) and decreased the genetic variance of z_3 ($\beta_{\sigma^2_a} = -0.65, p_- = 0.99$), with very weak statistical support for a positive effect on the genetic variance of z_2 ($\beta_{\sigma^2_a} = 0.22, p_+ = 0.73$); the second continuous covariate x_2 showed modest evidence for increasing the genetic variance of z_1 ($\beta_{\sigma^2_a} = 0.35, p_+ = 0.92$) and z_3 ($\beta_{\sigma^2_a} = 0.24, p_+ = 0.90$), with much stronger evidence that x_2 decreased the genetic variance of z_2 ($\beta_{\sigma^2_a} = -0.62, p_- = 0.98$); and the third genetic covariate x_3 increased the genetic variance of z_1 ($\beta_{\sigma^2_a} = 0.73, p_+ = 0.99$) and z_2 ($\beta_{\sigma^2_a} = 0.64, p_+ = 0.99$) but decreased the genetic variance of z_3 ($\beta_{\sigma^2_a} = -0.83, p_- = 1.00$).

The same approach can be taken to summarize RN parameters for genetic correlations, after appropriately transforming the RN parameters for canonical partial correlations from the Stan model using the custom `cor_beta_f()` function.

```
#generate beta coefficients for genetic correlations
cor_b = cor_beta_f(x = stan.dl$X, traits = traits, cpc_b = post$B_cpc)
head(cor_b, 3)
```

```
##   element X.Intercept.       X1          X2       X3
## 1   Z1_Z2      1.12865 -1.02048 -0.09013070 0.686910
## 2   Z1_Z2      1.10139 -1.05922 -0.24315200 0.693371
## 3   Z1_Z2      1.01191 -1.06465  0.00150996 0.914211
```

```
#posterior median
aggregate(.~ element, data = cor_b, FUN = function(x)
  round(median(x),2))
```

```
##   element X.Intercept.    X1    X2    X3
## 1   Z1_Z2         1.20 -1.13 -0.18  0.90
## 2   Z1_Z3        -0.74 -0.44 -0.41 -0.24
## 3   Z2_Z3        -0.70  0.40 -0.22 -0.45
```

```
#posterior probability in direction of posterior median
aggregate(.~ element, data = cor_b, FUN = function(x)
  sum(sign(median(x))==sign(x))/length(x))
```

```
##   element X.Intercept.     X1     X2     X3
## 1   Z1_Z2       0.9915 0.9990 0.7525 0.9735
## 2   Z1_Z3       0.9755 0.8870 0.8905 0.7150
## 3   Z2_Z3       0.9570 0.7575 0.6810 0.7685
```

```
#posterior 90% CIs
aggregate(.~ element, data = cor_b, FUN = function(x)
  round(quantile(x, c(0.05,0.95)),2), simplify = F)
```

```
##   element X.Intercept.           X1          X2          X3
## 1   Z1_Z2   0.38, 2.37 -2.04, -0.52 -0.68, 0.28  0.17, 1.78
## 2   Z1_Z3 -1.62, -0.13  -1.38, 0.17 -1.26, 0.21 -1.03, 0.75
## 3   Z2_Z3 -1.53, -0.04  -0.69, 1.27 -1.14, 0.61 -1.69, 0.68
```

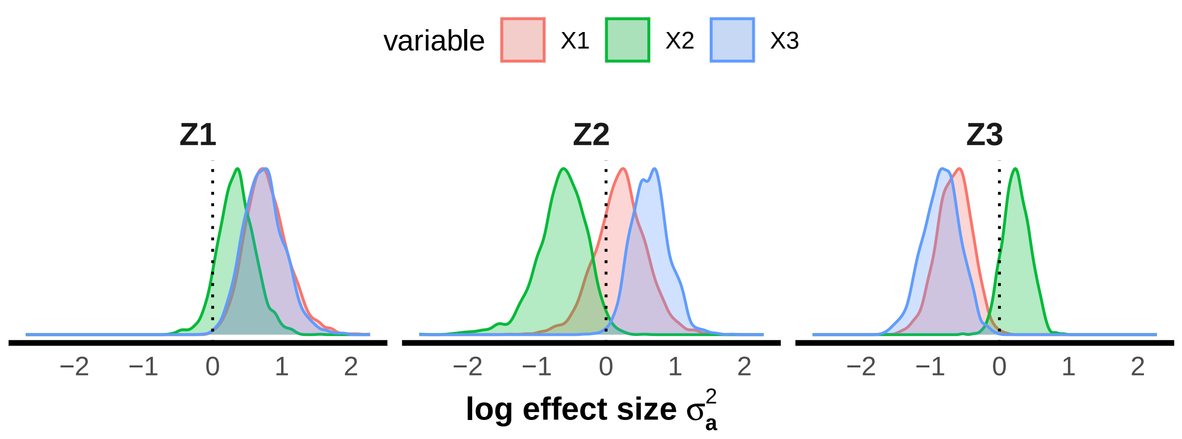
These effects are on the atanh scale. The first continuous covariate x_1 on average decreased the genetic correlation between z_1 and z_2 ($\beta_{r_a} = -1.13, p_- = 0.99$) with weaker evidence for decreasing the correlation between z_1 and z_3 ($\beta_{r_a} = -0.44, p_- = 0.89$) and increasing the correlation between z_2 and z_3 ($\beta_{r_a} = 0.40, p_+ = 0.76$); the second continuous covariate x_2 showed modest to weak support for decreasing the genetic correlation among all behaviors ($z_1 \sim z_2$: $\beta_{r_a} = -0.18, p_- = 0.75$; $z_1 \sim z_3$: $\beta_{r_a} = -0.41, p_- = 0.89$; $z_2 \sim z_3$: $\beta_{r_a} = -0.22, p_- = 0.68$); and the third covariate x_3 increased the genetic correlation among z_1 and z_2 ($\beta_{r_a} = 0.90, p_+ = 0.97$), with weak evidence for decreasing the genetic correlation among z_1 and z_3 ($\beta_{r_a} = -0.24, p_- = 0.72$) and z_2 and z_3 ($\beta_{r_a} = -0.45, p_- = 0.77$). The uncertainty of these effects is unsurprising, given that our simulation considers moderately sized effects of 3 environmental factors on 3 traits across 25 contexts. Increasing the number of contexts observed will increase power to detect multiple effects across contexts, as with any multivariate regression analysis.

Plot results

There are multiple ways to visualize the results of the analysis. One way is to simply plot the full posterior estimates of the $\beta_{\sigma^2_a}$ and β_{r_a} values. This can be easily accomplished with the outputs generated above using the `geom_density()` function from the ggplot2 package.

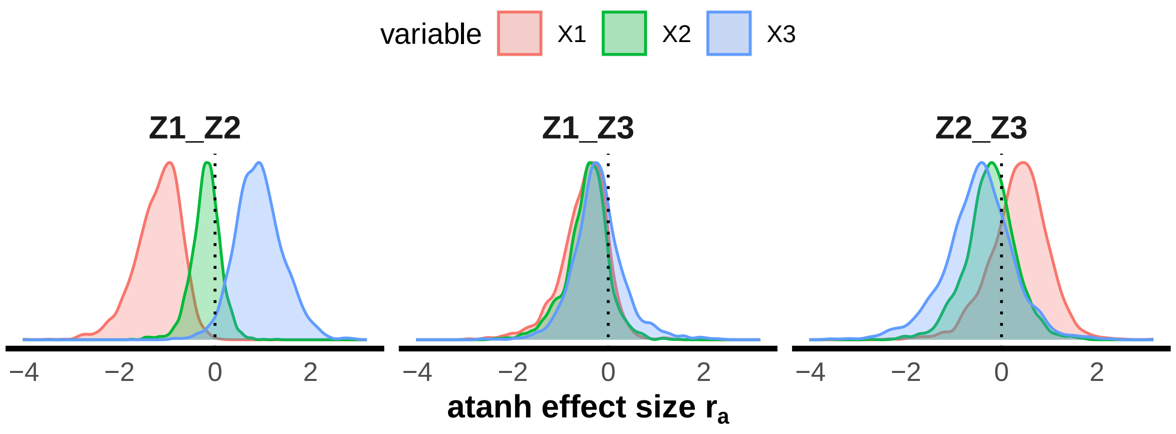
```
#long format
v_bl = melt(v_b)
v_bl2 = v_bl[v_bl$variable!="X.Intercept.", ] #excl intercepts

#plot posteriors of variance RNs
ggplot(v_bl2, aes(x = value, color = variable, fill = variable))+
  geom_density(aes(y = after_stat(scaled)), alpha = 0.30)+
  geom_vline(xintercept = 0, lty = "dotted")+
  facet_wrap(.~element)+
  labs(x=bquote(bold(paste("log effect size ", sigma[a]^2))))+
  theme(
    legend.position = "top",
    axis.ticks.x=element_blank(),
    axis.ticks.y=element_blank(),
    axis.title.x=element_text(size=12,face="bold"),
    axis.title.y = element_blank(),
    axis.text.x=element_text(size=10),
    axis.text.y=element_blank(),
    axis.line.x = element_line(linewidth = 1),
    axis.line.y = element_blank(),
    strip.text = element_text(size = 12, face = "bold"),
    strip.background = element_blank(),
    panel.background= element_blank(),
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(),
    plot.margin = unit(c(0.1,0.5,0.5,0.5), "cm"))+
    guides()
```



```
cor_bl = melt(cor_b)
cor_bl2 = cor_bl[cor_bl$variable!="X.Intercept.", ] #excl intercepts

#plot posteriors of variance RNs
ggplot(cor_bl2, aes(x = value, color = variable, fill = variable))+
  geom_density(aes(y = after_stat(scaled)), alpha = 0.30)+
  geom_vline(xintercept = 0, lty = "dotted")+
  facet_wrap(.~element)+
  labs(x=bquote(bold(paste("atanh effect size ", r[a]))))+
  theme(
    legend.position = "top",
    axis.ticks.x=element_blank(),
    axis.ticks.y=element_blank(),
    axis.title.x=element_text(size=12,face="bold"),
    axis.title.y = element_blank(),
    axis.text.x=element_text(size=10),
    axis.text.y=element_blank(),
    axis.line.x = element_line(linewidth = 1),
    axis.line.y = element_blank(),
    strip.text = element_text(size = 12, face = "bold"),
    strip.background = element_blank(),
    panel.background= element_blank(),
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(),
    plot.margin = unit(c(0.1,0.5,0.5,0.5), "cm"))+
    guides()
```

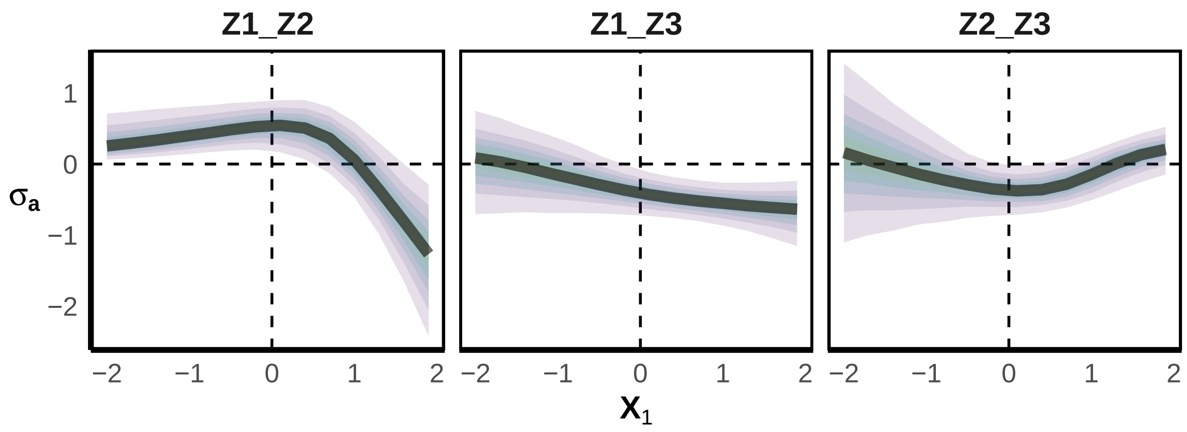


The posterior RNs for genetic variances and correlations can also be integrated together to predict and visualize how environmental contexts shape genetic (co)variances. In this case, we'll consider how genetic (co)variances are expected to change across the range [-2,+2] due to the marginal additive effect of environmental covariate x_1, holding x_2 and x_3 constant at their average values (0). The `stat_lineribbon()` function from the tidybayes package is used to visualize these posterior predictions as a CRN, with the dark line for the posterior median indicating the most probable curve and the accompanying ribbons of 10-90% credible intervals indicating statistical uncertainty around the median.

```
#predictor matrix for generating predictions from model
#X_pred columns must follow order of original design matrix
#colnames(stan.df$X), but the colnames of X_pred are arbitrary
seq = seq(-2,2, by = 0.3) #standardized values from -2 to +2
X_pred = data.frame(int = 1, X1 = seq, X2 = 0, X3 = 0)

#functions for predicting genetic correlations and covariances
mv_cpc = cpc_f(x = X_pred, cpc_b = post$B_cpc)
mv_cor = cor_f(x = X_pred, traits = traits,  cpc = mv_cpc)
mv_cov = cov_f(x = X_pred, traits = traits, v_b = post$B_v, cpc = mv_cpc)

#visualize change in covariance
levels = 6 #number of bands for Bayesian CIs, try ppoints(levels)
ggplot(mv_cov,
      aes(x = X1, y = value))+
      geom_hline(yintercept = 0, lty = "dashed")+
      geom_vline(xintercept = 0, lty = "dashed")+
      stat_lineribbon(size=2, .width = ppoints(levels), alpha=0.8/levels)+
      labs(x=bquote(bold(paste(X[1]))),
          y=bquote(bold(paste(sigma[a]))))+
      facet_wrap(.~ element)+
      theme(plot.title =element_text(size=12, face="bold",hjust=0.5),
        axis.ticks.y=element_blank(),
        axis.ticks.x=element_blank(),
        axis.title.x=element_text(size=12,face="bold"),
        axis.title.y=element_text(size=12,face="bold", angle = 0, vjust = 0.5),
        axis.text.x=element_text(size=10),
        axis.text.y=element_text(size=10),
        axis.line = element_line(linewidth = 1),
        panel.border=element_rect(fill=NA,color="black", linewidth=1,
                                  linetype="solid"),
        strip.text = element_text(size = 12, face = "bold"),
        strip.background = element_blank(),
        panel.background= element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        plot.margin = unit(c(0.1,0.5,0.5,0.5), "cm"))+
        guides(fill="none", color ="none")
```



This concludes the tutorial. The accompanying R and Stan files for the meerkat analysis provide a more advanced example showcasing the flexibility of Stan in handling complex data structures with repeated measures, heterogeneous sampling across measures, and non-Gaussian traits. Please contact me by email (jordan.martin@uzh.ch) if you have any questions or would like some assistance in adapting this code to your dataset.