

Preparing case-parent trio data and detecting disease-associated SNP interactions with `trio`

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1 Introduction

The R package `trio` contains functions for performing genotypic transmission disequilibrium tests (gTDTs) for testing whether the distributions of individual SNPs (Schaid, 1996) or two-way interactions of SNPs (Cordell, 2002; Cordell *et al.*, 2004) differ between the cases, i.e. the children, and the pseudo-controls derived from the parents' genotypes.

Furthremore, `trio` provides functionalities relevant for the analysis of case-parent trio data with *trio logic regression* (Li *et al.*, 2010). Two major features are implemented in this package: functions that aid in the transformation of the trio data from standard linkage files (ped format) or genotype format into objects suitable as input for trio logic regression, and a framework that allows for the simulation of case-parent data, where the risk of disease is specified by (higher order) SNP interactions.

In Section 2 of this vignette, it is shown how family-based data stored in a linkage/ped file can be read into R and transformed into a format suitable for the application of the functions for performing the genotypic TDTs, whereas Section 3 contains examples for the application of these gTDT functions to individual SNPs and two-way SNP interactions.

Section 4 is devoted to the steps relevant for data processing, to derive a matrix suitable as input for trio logic regression, starting from a linkage or genotype file which possibly

contains missing data and/or Mendelian errors. We give some examples how missing data can be addressed using haplotype-based imputation. The haplotype information can be specified by the user, or when this information is not readily available, automatically inferred. The haplotype blocks are also relevant in the delineation of the genotypes for the pseudo-controls, as the linkage disequilibrium (LD) structure observed in the parents is taken into account in this process. While this function is intended to generate complete case pseudo-control data as input for trio logic regression, an option to simply return the completed trio data is available.

For the estimation of the haplotype structure that might be used in the functions described in Section 4, the R package `trio` also contains functions for computing and plotting the pairwise LD values and for detecting LD blocks. In Section 5, it is described how these pairwise values of the typically used LD measures D' and r^2 can be computed with the function `getLD()`, and how the D' values can be employed to estimate haplotype blocks with the algorithm of Gabriel *et al.* (2002).

Finally, Section 6 of the vignette explains in more detail how to set up simulations of case-parent trio data, where the risk of disease is specified by SNP interactions. The most time-consuming step for these types of simulations is the generation of mating tables and the respective probabilities. The mating table information, however, can be stored, which allows for fast simulations when replicates of the case-parent trio data are generated.

2 Preparing data for the genotypic TDTs

Case-parent trio data are typically stored in a ped file. The first six columns in such a ped file, which is also called linkage file, identify the family structure of the data, and the phenotype. It is assumed that only one phenotype variable (column 6) is used. The object `trio.ped1`, available in the R package, is an example of a data set in ped format. It contains information for 10 SNPs in 100 trios. Besides the variables providing information

on the family structure and the phenotypes (columns 1–6), each SNP is encoded in two variables denoting the alleles.

```
> library(trio)
> data(trio.data)
> str(trio.ped1)

'data.frame':      300 obs. of  26 variables:
 $ famid   : int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid     : int   1 2 3 1 2 3 1 2 3 1 ...
 $ fatid   : int   0 0 1 0 0 1 0 0 1 0 ...
 $ motid   : int   0 0 2 0 0 2 0 0 2 0 ...
 $ sex     : int   1 2 2 1 2 1 1 2 1 1 ...
 $ affected: int   0 0 2 0 0 2 0 0 2 0 ...
 $ snp1_1  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp1_2  : int   1 1 1 1 1 1 1 1 1 2 ...
 $ snp2_1  : int   1 2 1 1 2 1 1 1 1 1 ...
 $ snp2_2  : int   1 2 2 1 2 2 1 1 1 1 ...
 $ snp3_1  : int   1 1 1 2 1 1 1 1 1 1 ...
 $ snp3_2  : int   2 1 2 2 1 2 1 2 1 2 ...
 $ snp4_1  : int   1 1 1 2 1 1 1 1 1 1 ...
 $ snp4_2  : int   2 1 2 2 1 2 1 2 1 2 ...
 $ snp5_1  : int   1 2 1 1 2 1 1 1 1 1 ...
 $ snp5_2  : int   2 2 2 1 2 2 2 1 1 1 ...
 $ snp6_1  : int   1 1 1 2 1 1 1 1 1 1 ...
 $ snp6_2  : int   2 1 2 2 1 2 1 2 1 2 ...
 $ snp7_1  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp7_2  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp8_1  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp8_2  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp9_1  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp9_2  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp10_1 : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp10_2 : int   1 1 1 2 1 1 1 1 1 2 ...

> trio.ped1[1:10,1:12]
```

	famid	pid	fatid	motid	sex	affected	snp1_1	snp1_2	snp2_1	snp2_2	snp3_1	snp3_2
1	10001	1	0	0	1	0	1	1	1	1	1	2
2	10001	2	0	0	2	0	1	1	2	2	1	1
3	10001	3	1	2	2	2	1	1	1	2	1	2
4	10002	1	0	0	1	0	1	1	1	1	2	2
5	10002	2	0	0	2	0	1	1	2	2	1	1
6	10002	3	1	2	1	2	1	1	1	2	1	2
7	10003	1	0	0	1	0	1	1	1	1	1	1
8	10003	2	0	0	2	0	1	1	1	1	1	2
9	10003	3	1	2	1	2	1	1	1	1	1	1
10	10004	1	0	0	1	0	1	2	1	1	1	2

If not already available as data frame or matrix in the R workspace, trio data can be read into R using the function `read.pedfile()`. If we, for example, assume that the working directory of the current R session contains a file called "pedfile.ped" (this file is actually not available in `trio`, we just assume that such a file exists in the working directory), then this file can be read into R by calling

```
> ped <- read.pedfile("pedfile.ped")
```

If the arguments `coded` and `first.row` of `read.pedfile()` are not specified by the user, `read.pedfile()` automatically tries to figure out how the alleles in the ped file are coded, and whether the first row contains the SNP names (`first.row = FALSE`) or the data for the first subject (`first.row = TRUE`). In the former case, `read.pedfile()` adds the SNP names (with extensions `.1` and `.2` to differ between the two alleles) to the respective columns of the read data frame.

For the applications of the functions for performing gTDTs (see Section 3), the trio data must be in a matrix in genotype format. In such a matrix, each column represents a SNP, which is coded by the number of minor alleles, and each block of 3 consecutive rows contains the genotypes of the father, the mother, and their offspring (in this order) of one specific trio. Missing values are allowed in this matrix, and need to be coded by `NA`. This matrix can either be generated from a data frame in ped format by employing the function `ped2geno()`, or more conveniently, by setting `p2g = TRUE` in `read.pedfile()`. Thus, a matrix in genotype format might be obtained from the above ped file by calling

```
> geno <- read.pedfile("pedfile.ped", p2g=TRUE)
```

The output of these functions just contains the matrix in genotype format, whereas `trio.check()` described in Section 4 additionally contains information about Mendelian

errors. Instead of checking for Mendelian errors in `ped2geno()` or `read.pedfile()`, such errors are removed SNP-wise in the functions for performing genotypic TDTs.

If, for example, the data frame `trio.ped1` should be transformed into a matrix in genotype format, `ped2geno()` can be applied to it. However, `ped2geno()` requires unique personal IDs (second column of `trio.ped1`) such that we first have to combine the family ID and the personal ID (which would be automatically done by `read.pedfile()`), and change the IDs of the fathers and mothers in columns 3 and 4 likewise.

```
> data(trio.data)
> trio.ped1[,2] <- paste(trio.ped1[,1], trio.ped1[,2], sep="_")
> ids <- trio.ped1[,3] != 0
> trio.ped1[ids,3] <- paste(trio.ped1[ids,1], trio.ped1[ids,3], sep="_")
> trio.ped1[ids,4] <- paste(trio.ped1[ids,1], trio.ped1[ids,4], sep="_")
> trio.ped1[1:5, 1:4]
```

	famid	pid	fatid	motid
1	10001	10001_1	0	0
2	10001	10001_2	0	0
3	10001	10001_3	10001_1	10001_2
4	10002	10002_1	0	0
5	10002	10002_2	0	0

Afterwards, `ped2geno()` can be applied to `trio.ped1`

```
> geno <- ped2geno(trio.ped1)
> geno[1:5,]
```

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10
10001_1	0	0	1	1	1	1	0	0	0	0
10001_2	0	2	0	0	2	0	0	0	0	0
10001_3	0	1	1	1	1	1	0	0	0	0
10002_1	0	0	2	2	0	2	0	0	0	1
10002_2	0	2	0	0	2	0	0	0	0	0

The matrix `trio.gen1` is the genotype matrix corresponding to `trio.ped1`. So the genotypes in the output of `ped2geno()` are identical to `trio.gen1` (except that the first two columns of `trio.gen1` contain the family ID and the personal ID).

```
> data(trio.data)
> trio.gen1[1:5, 3:12]
```

	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
1	0	0	1	1	1	1	0	0	0	0
2	0	2	0	0	2	0	0	0	0	0
3	0	1	1	1	1	1	0	0	0	0
4	0	0	2	2	0	2	0	0	0	1
5	0	2	0	0	2	0	0	0	0	0

```
> table(trio.gen1[,3:12] == geno)
```

```
TRUE
3000
```

3 Testing SNPs and two-way SNP interactions

A single SNP or a two-way interaction can be tested with a gTDT by employing the functions `tdt()` and `tdt2way()`. If we, for example, would like to test the first SNP in the matrix `mat.test` available in the R package `trio`, then this could be done by calling

```
> data(trio.data)
> tdt(mat.test[,1])
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

Coef	OR	Lower	Upper	SE	Statistic	p-Value
-0.04256	0.9583	0.6396	1.436	0.2063	-0.2063	0.8366

In this case, a conditional logistic regression is fitted, and the output of `tdt()` contains the parameter estimate `Coef` for the SNP in this model, the odds ratio `OR`, the `Lower` and `Upper` bound of the 95% confidence interval of this odds ratio, the standard error `SE` of the parameter estimate, the Wald `Statistic` for testing whether this SNP has an effect, and the corresponding `p-Value`.

By default, an additive effect is assumed. It is, however, also possible to consider a dominant effect

```
> tdt(mat.test[,1], model="dominant")
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Dominant

Coef	OR	Lower	Upper	SE	Statistic	p-Value
-0.1134	0.8928	0.5219	1.527	0.2739	-0.4139	0.679

or a recessive effect

```
> tdt(mat.test[,1], model="recessive")
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Recessive

Coef	OR	Lower	Upper	SE	Statistic	p-Value
0.06502	1.067	0.5279	2.157	0.3591	0.1810	0.8563

Similarly the interaction between SNP1 and SNP2 in `mat.test` can be tested by

```
> tdt2way(mat.test[,1], mat.test[,2])
```

Genotypic TDT for Epistatic Interactions (Using 15 Pseudo Controls)

Likelihood Ratio Test:

Loglikelihood (with Interactions): -272.644

Loglikelihood (without IAs): -275.29

Test Statistic: 5.293

P-Value: 0.26

In this case, the interaction is tested for epistatic interactions as described in Cordell (2002). Thus, two conditional logistic regression models are fitted, one consisting of two dummy variables for each of the two SNPs, and the other additionally containing the four possible interactions of these dummy variables. The two fitted models are then compared by a likelihood ratio test, and the p -values are computed by approximation to a χ^2 -distribution with four degrees of freedom.

This is the recommended way to test the two-way interaction. `tdt2way()`, however, also provides a simpler test, in which the values of the SNPs (either coding for an additive – which is the default – or for a recessive, or for a dominant model) are multiplied, and a conditional logistic regression is applied to this interaction.

```
> tdt2way(mat.test[,1], mat.test[,2], epistatic=FALSE)
```

Genotypic TDT for Two-Way Interaction (Using 15 Pseudo Controls)

Model Type: Additive

	Coef	OR	Lower	Upper	SE	Statistic	p-Value
	0.02424	1.025	0.7806	1.345	0.1387	0.1747	0.8613

All SNPs represented by the columns of a matrix in genotype format can be tested with a gTDT by employing the function `colTDT()`. Thus, all SNPs in `mat.test` can be tested by calling

```
> tdt.out <- colTDT(mat.test)
> tdt.out
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

Top 5 SNPs:

	Coef	OR	Lower	Upper	SE	Statistic	p-Value
SNP6	0.44895	1.5667	0.9910	2.477	0.2337	1.9212	0.05471
SNP3	-0.22884	0.7955	0.5103	1.240	0.2265	-1.0104	0.31232
SNP2	-0.19671	0.8214	0.5561	1.213	0.1990	-0.9886	0.32288
SNP4	-0.13353	0.8750	0.5783	1.324	0.2113	-0.6320	0.52740
SNP5	0.09764	1.1026	0.7148	1.701	0.2211	0.4416	0.65881

By default, the five top SNPs, i.e. the five SNPs with the lowest p -values, are shown ordered by their significance. The top three SNPs are shown by

```
> print(tdt.out, 3)
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

Top 3 SNPs:

	Coef	OR	Lower	Upper	SE	Statistic	p-Value
SNP6	0.4490	1.5667	0.9910	2.477	0.2337	1.9212	0.05471
SNP3	-0.2288	0.7955	0.5103	1.240	0.2265	-1.0104	0.31232
SNP2	-0.1967	0.8214	0.5561	1.213	0.1990	-0.9886	0.32288

If the integer specified in `print()` is larger than or equal to the number of SNPs in the input matrix, the statistics for all SNPs are displayed in the order of their appearance in

this matrix.

```
> print(tdt.out, 10)
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

	Coef	OR	Lower	Upper	SE	Statistic	p-Value
SNP1	-0.04256	0.9583	0.6396	1.436	0.2063	-0.2063	0.83658
SNP2	-0.19671	0.8214	0.5561	1.213	0.1990	-0.9886	0.32288
SNP3	-0.22884	0.7955	0.5103	1.240	0.2265	-1.0104	0.31232
SNP4	-0.13353	0.8750	0.5783	1.324	0.2113	-0.6320	0.52740
SNP5	0.09764	1.1026	0.7148	1.701	0.2211	0.4416	0.65881
SNP6	0.44895	1.5667	0.9910	2.477	0.2337	1.9212	0.05471

Analogously, `trio` provides a function `colTDT2way()` for testing two-way interactions. Since this `gTDT` assumes that the considered loci are unlinked, the testing might fail, i.e. the fitting of at least one of the two conditional logistic regressions might not work properly, if SNPs are in (strong) LD. (Another reason why the fitting might not work properly is that the minor allele frequencies of both SNPs are small.) Therefore, `colTDT2way()` provides an argument called `genes` that allows specifying which SNP belongs to which LD-block, gene, or genetic region. If `genes` is not specified, the interactions between all $m(m-1)/2$ pairs of the m SNPs in a matrix are tested. If specified, only the interactions between SNPs showing different values of `genes` are tested.

If we thus assume that the first two SNPs in `mat.test` belong to gene G1 and the other four SNPs to G2

```
> genes <- paste("G", rep(1:2, c(2,4)), sep="")
> genes

[1] "G1" "G1" "G2" "G2" "G2" "G2"
```

then only the four interactions between SNP1 and each SNP from gene G2, as well as the four interactions between SNP2 and each SNP from gene G2 are tested, when calling

```
> tdt2.out <- colTDT2way(mat.test, genes=genes)
> tdt2.out
```

Genotypic TDT for Epistatic Interactions (Using 15 Pseudo Controls)

Top 5 SNP Interactions (Likelihood Ratio Test):

	LL (with IAs)	LL (w/o IAs)	Statistic	P-Value	Genes
SNP1 : SNP5	-269.5	-277.0	15.069	0.004561	G1 : G2
SNP2 : SNP4	-270.3	-275.0	9.528	0.049167	G1 : G2
SNP1 : SNP3	-273.0	-275.2	4.440	0.349724	G1 : G2
SNP2 : SNP5	-273.3	-275.3	3.871	0.423763	G1 : G2
SNP2 : SNP6	-271.2	-272.6	2.805	0.591008	G1 : G2

Again, by default the top five SNP interactions are shown. The statistics for all eight interactions can be displayed by calling

```
> print(tdt2.out, 8)
```

Genotypic TDT for Epistatic Interactions (Using 15 Pseudo Controls)

Likelihood Ratio Test:

	LL (with IAs)	LL (w/o IAs)	Statistic	P-Value	Genes
SNP1 : SNP3	-273.0	-275.2	4.440	0.349724	G1 : G2
SNP1 : SNP4	-275.9	-276.8	1.653	0.799239	G1 : G2
SNP1 : SNP5	-269.5	-277.0	15.069	0.004561	G1 : G2
SNP1 : SNP6	-273.3	-274.3	2.050	0.726494	G1 : G2
SNP2 : SNP3	-273.0	-273.4	0.778	0.941371	G1 : G2
SNP2 : SNP4	-270.3	-275.0	9.528	0.049167	G1 : G2
SNP2 : SNP5	-273.3	-275.3	3.871	0.423763	G1 : G2
SNP2 : SNP6	-271.2	-272.6	2.805	0.591008	G1 : G2

4 Generating data for trio logic regression input

If interactions of a higher order than two are of interest, trio logic regression can be used to detect disease-associated SNP interactions of any order.

To generate data that can be used as input in trio logic regression, the sequential application of two functions is required. The function `trio.check()` evaluates whether or not Mendelian errors are present in the data (stored either in linkage or in genotype format, see Section 4.1). If no Mendelian inconsistencies are detected, this function creates an object that is passed to the function `trio()`. The latter function then generates a matrix of the genotype information for the affected probands and the inferred pseudo-controls,

taking the observed LD structure into account. Missing data are imputed in the process. The user, however, has to supply the information for the lengths of the LD blocks. A function called `findLDblocks()` for identifying LD blocks, and thus, for specifying the length of the blocks is therefore also contained in this package (see Section 5). Given the lengths of the LD blocks, the haplotype frequencies can be estimated, using the function `haplo.em()` in the `haplo.stat` package.

4.1 Supported file formats and elementary data processing

In this section, we show how to generate data suitable for input to trio logic regression from complete pedigree data without Mendelian errors. The function `trio.check()` requires that the trio data are already available as a data frame or matrix, either in linkage/ped format (the default), or in genotype format (for reading a ped file into R, see Section 3).

The first function used is always `trio.check()`. Unless otherwise specified, this function assumes that the data are in linkage format. If no Mendelian inconsistencies in the data provided are identified, `trio.check()` creates an object that can be processed in the subsequent analysis with this package. The genotype information for each SNP will be converted into a single variable, denoting the number of variant alleles.

If we thus would like to check whether the data frame `trio.ped1` contains Mendelian errors, we call

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.ped1)
> str(trio.tmp, max=1)

List of 2
 $ trio :'data.frame':      300 obs. of  12 variables:
  $ errors: NULL

> trio.tmp$trio[1:6,]

  famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
1 10001   1    0    0    1    1    1    1    0    0    0    0
```

2	10001	2	0	2	0	0	2	0	0	0	0	0
3	10001	3	0	1	1	1	1	1	0	0	0	0
4	10002	1	0	0	2	2	0	2	0	0	0	1
5	10002	2	0	2	0	0	2	0	0	0	0	0
6	10002	3	0	1	1	1	1	1	0	0	0	0

Taking the LD structure of the SNPs into account is imperative when creating the genotypes for the pseudo-controls. This requires information on the LD blocks. However, there are many ways to delineate this block structure, and in the absence of a consensus what the best approach is, researchers have different preferences, and thus, results can be different. In the function `findLDblocks()`, a modified version of the method of Gabriel *et al.* (2002) has been implemented, which can be used to specify the block structure by

```
> table(foundBlocks$blocks)
```

if `foundBlocks` is the output of `findLDblocks()` (for details, see Section 5).

The function `trio()`, which operates on an output object of `trio.check()`, accepts the block length information as an argument (in the following, we assume that the block structure is given by `c(1, 4, 2, 3)`, i.e. the first block consists only of the first SNP, the second block of the next four SNPs, the third of the following two SNPs, and the last block of the remaining three SNPs). If this argument is not specified, a uniform block length of 1 (i.e. no LD structure) is assumed. If the haplotype frequencies are not specified, they are estimated from the parents' genotypes (more information on this in the following sections). The function `trio()` then returns a list that contains the genotype information in binary format, suitable as input for trio logic regression: `bin` is a matrix with the conditional logistic regression response in the first columns, and each SNP as two binary variables using dominant and recessive coding. The list element `miss` contains information about missing values in the original data, and `freq` contains information on the estimated haplotype frequencies.

```
> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> str(trio.bin, max=1)
```

```

List of 3
 $ bin : num [1:400, 1:21] 3 0 0 0 3 0 0 0 3 0 ...
  ..- attr(*, "dimnames")=List of 2
 $ miss: NULL
 $ freq:'data.frame':      19 obs. of  3 variables:

> trio.bin$bin[1:8,]

      y snp1.D snp1.R snp2.D snp2.R snp3.D snp3.R snp4.D snp4.R snp5.D snp5.R
[1,] 3      0      0      1      0      1      0      1      0      1      0
[2,] 0      0      0      1      0      0      0      0      0      1      1
[3,] 0      0      0      1      0      0      0      0      0      1      1
[4,] 0      0      0      1      0      1      0      1      0      1      0
[5,] 3      0      0      1      0      1      0      1      0      1      0
[6,] 0      0      0      1      0      1      0      1      0      1      0
[7,] 0      0      0      1      0      1      0      1      0      1      0
[8,] 0      0      0      1      0      1      0      1      0      1      0
      snp6.D snp6.R snp7.D snp7.R snp8.D snp8.R snp9.D snp9.R snp10.D snp10.R
[1,]      1      0      0      0      0      0      0      0      0      0
[2,]      1      0      0      0      0      0      0      0      0      0
[3,]      0      0      0      0      0      0      0      0      0      0
[4,]      0      0      0      0      0      0      0      0      0      0
[5,]      1      0      0      0      0      0      0      0      0      0
[6,]      1      0      0      0      0      0      0      0      1      0
[7,]      1      0      0      0      0      0      0      0      1      0
[8,]      1      0      0      0      0      0      0      0      0      0

```

As mentioned above, the *trio* package also accommodates trio genotype data. The object *trio.gen1*, available in the R package, is an example of such a data set. Equivalent to *trio.ped1* used above, it contains information for 10 SNPs in 100 trios. When used in *trio.check()*, the argument *is.linkage* needs to be set to *FALSE*. The output from this function is then identical to the one shown derived from the linkage file, and can be passed to the function *trio()*.

```

> data(trio.data)
> str(trio.gen1)

'data.frame':      300 obs. of  12 variables:
 $ famid: int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid  : int   1 2 3 1 2 3 1 2 3 1 ...
 $ snp1 : int   0 0 0 0 0 0 0 0 0 1 ...
 $ snp2 : int   0 2 1 0 2 1 0 0 0 0 ...
 $ snp3 : int   1 0 1 2 0 1 0 1 0 1 ...
 $ snp4 : int   1 0 1 2 0 1 0 1 0 1 ...
 $ snp5 : int   1 2 1 0 2 1 1 0 0 0 ...
 $ snp6 : int   1 0 1 2 0 1 0 1 0 1 ...

```

```

$ snp7 : int  0 0 0 0 0 0 0 0 0 0 ...
$ snp8 : int  0 0 0 0 0 0 0 0 0 0 ...
$ snp9 : int  0 0 0 0 0 0 0 0 0 0 ...
$ snp10: int  0 0 0 1 0 0 0 0 0 1 ...

> trio.gen1[1:10,1:12]

      famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
1  10001   1    0    0    1    1    1    1    0    0    0    0
2  10001   2    0    2    0    0    2    0    0    0    0    0
3  10001   3    0    1    1    1    1    1    0    0    0    0
4  10002   1    0    0    2    2    0    2    0    0    0    1
5  10002   2    0    2    0    0    2    0    0    0    0    0
6  10002   3    0    1    1    1    1    1    0    0    0    0
7  10003   1    0    0    0    0    1    0    0    0    0    0
8  10003   2    0    0    1    1    0    1    0    0    0    0
9  10003   3    0    0    0    0    0    0    0    0    0    0
10 10004   1    1    0    1    1    0    1    0    0    0    1

> trio.tmp <- trio.check(dat=trio.gen1, is.linkage=F)
> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))

```

4.2 Missing genotype information

Missing genotypes in `ped(igree)` files are typically encoded using the integer 0. The data files can be processed as before if they contain such missing values:

```

> data(trio.data)
> str(trio.ped2)

'data.frame':      300 obs. of  26 variables:
 $ famid   : int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid     : int   1 2 3 1 2 3 1 2 3 1 ...
 $ fatid   : int   0 0 1 0 0 1 0 0 1 0 ...
 $ motid   : int   0 0 2 0 0 2 0 0 2 0 ...
 $ sex     : int   1 2 2 1 2 1 1 2 1 1 ...
 $ affected: int   0 0 2 0 0 2 0 0 2 0 ...
 $ snp1_1  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp1_2  : int   1 1 1 1 1 1 1 1 1 2 ...
 $ snp2_1  : int   1 0 1 1 2 1 1 1 1 1 ...
 $ snp2_2  : int   1 0 2 1 2 2 1 1 1 1 ...
 $ snp3_1  : int   1 1 1 2 0 1 1 1 1 1 ...
 $ snp3_2  : int   2 1 2 2 0 2 1 2 1 2 ...
 $ snp4_1  : int   1 0 1 2 1 1 1 1 1 1 ...
 $ snp4_2  : int   2 0 2 2 1 2 1 2 1 2 ...
 $ snp5_1  : int   1 2 1 1 2 1 1 1 1 1 ...

```

```

$ snp5_2 : int 2 2 2 1 2 2 2 1 1 1 ...
$ snp6_1 : int 1 1 1 0 1 1 1 1 1 1 ...
$ snp6_2 : int 2 1 2 0 1 2 1 2 1 2 ...
$ snp7_1 : int 1 1 1 1 1 1 1 1 0 1 ...
$ snp7_2 : int 1 1 1 1 1 1 1 1 0 1 ...
$ snp8_1 : int 1 1 1 1 0 1 1 1 0 1 ...
$ snp8_2 : int 1 1 1 1 0 1 1 1 0 1 ...
$ snp9_1 : int 1 1 1 1 1 1 1 0 1 1 ...
$ snp9_2 : int 1 1 1 1 1 1 1 0 1 1 ...
$ snp10_1 : int 1 1 1 1 1 1 1 1 1 1 ...
$ snp10_2 : int 1 1 1 2 1 1 1 1 1 2 ...

> trio.tmp <- trio.check(dat=trio.ped2)
> trio.tmp$trio[1:6,]

  famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
1 10001  1    0    0    1    1    1    1    0    0    0    0
2 10001  2    0   NA    0   NA    2    0    0    0    0    0
3 10001  3    0    1    1    1    1    1    0    0    0    0
4 10002  1    0    0    2    2    0   NA    0    0    0    1
5 10002  2    0    2   NA    0    2    0    0   NA    0    0
6 10002  3    0    1    1    1    1    1    0    0    0    0

```

Since trio logic regression requires complete data, the function `trio()` also performs an imputation of the missing genotypes. The imputation is based on estimated haplotypes, using the block length information specified by the user. In a later section we demonstrate how this imputation can be run more efficiently when haplotype frequency estimates are already available.

```

> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> trio.bin$bin[1:8,]

  y snp1.D snp1.R snp2.D snp2.R snp3.D snp3.R snp4.D snp4.R snp5.D snp5.R
[1,] 3    0    0    1    0    1    0    1    0    1    0
[2,] 0    0    0    1    0    1    0    1    0    1    0
[3,] 0    0    0    1    0    0    0    0    0    1    1
[4,] 0    0    0    1    0    0    0    0    0    1    1
[5,] 3    0    0    1    0    1    0    1    0    1    0
[6,] 0    0    0    1    0    1    0    1    0    1    0
[7,] 0    0    0    1    0    1    0    1    0    1    0
[8,] 0    0    0    1    0    1    0    1    0    1    0
  snp6.D snp6.R snp7.D snp7.R snp8.D snp8.R snp9.D snp9.R snp10.D snp10.R
[1,]  1    0    0    0    0    0    0    0    0    0
[2,]  1    0    0    0    0    0    0    0    0    0
[3,]  0    0    0    0    0    0    0    0    0    0
[4,]  0    0    0    0    0    0    0    0    0    0

```

```

[5,] 1 0 0 0 0 0 0 0 0 0 0
[6,] 1 0 0 0 0 0 0 0 0 0 0
[7,] 1 0 0 0 0 0 0 0 0 1 0
[8,] 1 0 0 0 0 0 0 0 0 1 0

```

Missing data in genotypes files should be encoded using NA, the conventional symbol in R to indicate missing values.

```

> data(trio.data)
> str(trio.gen2)

'data.frame':      300 obs. of  12 variables:
 $ famid: int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid  : int  1 2 3 1 2 3 1 2 3 1 ...
 $ snp1 : int  0 0 0 0 0 0 0 0 0 1 ...
 $ snp2 : int  0 2 1 NA NA 1 0 0 0 0 ...
 $ snp3 : int  1 NA 1 2 0 1 0 NA 0 1 ...
 $ snp4 : int  1 0 1 NA 0 1 0 1 0 1 ...
 $ snp5 : int  1 2 1 0 2 1 1 NA 0 0 ...
 $ snp6 : int  1 0 1 NA 0 1 0 1 0 1 ...
 $ snp7 : int  0 0 0 0 0 0 0 0 0 0 ...
 $ snp8 : int  0 0 NA 0 0 0 0 NA 0 0 ...
 $ snp9 : int  0 0 0 0 0 0 0 0 0 0 ...
 $ snp10: int  0 0 0 1 0 0 0 0 0 1 ...

> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> trio.bin$bin[1:8,]

```

```

      y snp1.D snp1.R snp2.D snp2.R snp3.D snp3.R snp4.D snp4.R snp5.D snp5.R
[1,] 3      0      0      1      0      1      0      1      0      1      0
[2,] 0      0      0      1      0      1      0      1      0      1      0
[3,] 0      0      0      1      0      0      0      0      0      1      1
[4,] 0      0      0      1      0      0      0      0      0      1      1
[5,] 3      0      0      1      0      1      0      1      0      1      0
[6,] 0      0      0      1      0      1      0      1      0      1      0
[7,] 0      0      0      0      0      1      0      1      0      1      0
[8,] 0      0      0      0      0      1      0      1      0      1      0
      snp6.D snp6.R snp7.D snp7.R snp8.D snp8.R snp9.D snp9.R snp10.D snp10.R
[1,] 1      0      0      0      0      0      0      0      0      0
[2,] 1      0      0      0      0      0      0      0      0      0
[3,] 0      0      0      0      0      0      0      0      0      0
[4,] 0      0      0      0      0      0      0      0      0      0
[5,] 1      0      0      0      0      0      0      0      0      0
[6,] 1      0      0      0      0      0      0      0      1      0
[7,] 1      0      0      0      0      0      0      0      0      0
[8,] 1      0      0      0      0      0      0      0      1      0

```


As the user might also be interested in the completed genotype data in the original format (genotype or linkage file), the function `trio()` also allows for this option by using the argument `logic = FALSE`. In the resulting object, the matrix `bin` is then replaced by the data frame `trio`, and `miss` and `freq` are also returned.

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> trio.imp <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=FALSE)
> str(trio.imp, max=1)
```

List of 3

```
$ trio:'data.frame':      300 obs. of  12 variables:
$ miss:'data.frame':      250 obs. of  5 variables:
$ freq:'data.frame':       19 obs. of  3 variables:
```

```
> trio.imp$miss[c(1:6),]
```

	famid	pid	snp	r	c
1	10001	2	3	2	5
2	10001	3	8	3	10
3	10002	1	2	4	4
4	10002	1	4	4	6
5	10002	1	6	4	8
6	10002	2	2	5	4

```
> print(trio.gen2[1:6,])
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
1	10001	1	0	0	1	1	1	1	0	0	0	0
2	10001	2	0	2	NA	0	2	0	0	0	0	0
3	10001	3	0	1	1	1	1	1	0	NA	0	0
4	10002	1	0	NA	2	NA	0	NA	0	0	0	1
5	10002	2	0	NA	0	0	2	0	0	0	0	0
6	10002	3	0	1	1	1	1	1	0	0	0	0

```
> print(trio.imp$trio[1:6,])
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
1	10001	1	0	0	1	1	1	1	0	0	0	0
2	10001	2	0	2	0	0	2	0	0	0	0	0
3	10001	3	0	1	1	1	1	1	0	0	0	0
4	10002	1	0	0	2	2	0	2	0	0	0	1
5	10002	2	0	1	0	0	2	0	0	0	0	0
6	10002	3	0	1	1	1	1	1	0	0	0	0

The same applies to pedigree data:

```

> data(trio.data)
> trio.tmp <- trio.check(dat=trio.ped2)
> trio.imp <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=FALSE)

```

4.3 Mendelian errors

To delineate the genotype information for the pseudo-controls, the trio data must not contain any Mendelian errors. The function `trio.check()` returns a warning, and an R object with relevant information when Mendelian errors are encountered is created.

```

> data(trio.data)
> trio.tmp <- trio.check(dat=trio.ped.err)

[1] "Found Mendelian error(s)."
```

```

> str(trio.tmp, max=1)

List of 3
 $ trio      : NULL
 $ errors    :'data.frame':      4 obs. of  5 variables:
 $ trio.err  :'data.frame':    300 obs. of 12 variables:

> trio.tmp$errors

   trio famid snp r  c
1     1  10001  9 1 11
2     1  10001 10 1 12
3     2  10002 10 4 12
4     3  10003 10 7 12

```

In this data set, trio 1, for example, contains two Mendelian errors, in SNPs 9 and 10.

```

> trio.tmp$trio.err[1:3, c(1,2, 11:12)]

   famid pid snp9 snp10
1 10001   1    0     1
2 10001   2    0     2
3 10001   3    2     0
```

```

> trio.ped.err[1:3,c(1:2, 23:26)]

   famid pid snp9_1 snp9_2 snp10_1 snp10_2
1 10001   1     1     1     1     2
2 10001   2     1     1     2     2
3 10001   3     2     2     1     1

```

It is the user's responsibility to find the cause for the Mendelian errors and correct those, if possible. However, Mendelian inconsistencies are often due to genotyping errors and thus, it might not be possible to correct those in a very straight-forward manner. In this instance, the user might want to encode the genotypes that cause these Mendelian errors in some of the trios as missing data. The argument `replace = TRUE` in `trio.check()` allows for this possibility. The resulting missing data can then be imputed as described in the previous section.

```
> trio.rep <- trio.check(dat=trio.ped.err, replace=TRUE)
> str(trio.rep, max=1)

List of 2
 $ trio  :'data.frame':      300 obs. of  12 variables:
 $ errors: NULL

> trio.rep$trio[1:3,11:12]

      snp9 snp10
1      NA      NA
2      NA      NA
3      NA      NA
```

The same option is available for data in genotype format with Mendelian inconsistencies.

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.gen.err, is.linkage=FALSE)

[1] "Found Mendelian error(s)."
```

```
> trio.tmp$errors

      trio famid snp r c
1       1  2001   5 1 7
2       2  2002   5 4 7
```

```
> trio.tmp$trio.err[1:6, c(1,2,7), drop=F]

      famid pid snp5
6    2001   1     0
7    2001   2     0
5    2001   3     1
9    2002   1     1
10   2002   2     0
8    2002   3     2
```

```
> trio.rep <- trio.check(dat=trio.gen.err, is.linkage=FALSE, replace=TRUE)
> trio.rep$trio[1:6,c(1,2,7)]
```

```
      famid pid snp5
6      2001   1  NA
7      2001   2  NA
5      2001   3  NA
9      2002   1  NA
10     2002   2  NA
8      2002   3  NA
```

4.4 Using haplotype frequencies

As mentioned above, when estimates for the haplotype frequencies are already available, they can be used in the imputation of missing data and the delineation of the pseudo-controls. In case there are blocks of length one, i.e. SNPs not belonging to any LD blocks, the minor allele frequencies of those SNPs are supplied. In this case, no haplotype estimation is required when the function `trio()` is run, which can result in substantial time savings.

As an example for the format of a file containing haplotype frequency estimates and SNP minor allele frequencies, the object `freq.hap` is available in the R package:

```
> data(trio.data)
> str(freq.hap)

'data.frame':      20 obs. of  3 variables:
 $ key : int  1 1 2 2 2 2 2 2 2 3 ...
 $ hap : int  1 2 1111 1112 1121 1221 1222 2112 2222 11 ...
 $ freq: num  0.8 0.2 0.33734 0.20593 0.0024 ...

> freq.hap[1:6,]

      key  hap      freq
1      1    1 0.800000000
2      1    2 0.200000000
3      2 1111 0.337339745
4      2 1112 0.205929486
5      2 1121 0.002403846
6      2 1221 0.368589742
```

We can now impute the missing genotypes using these underlying haplotype frequencies.

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> trio.imp <- trio(trio.dat=trio.tmp, freq=freq.hap, logic=FALSE)
> str(trio.imp, max=1)
```

List of 3

```
$ trio:'data.frame':      300 obs. of  12 variables:
$ miss:'data.frame':      250 obs. of  5 variables:
$ freq:'data.frame':      20 obs. of  3 variables:
```

```
> print(trio.gen2[1:6,])
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
1	10001	1	0	0	1	1	1	1	0	0	0	0
2	10001	2	0	2	NA	0	2	0	0	0	0	0
3	10001	3	0	1	1	1	1	1	0	NA	0	0
4	10002	1	0	NA	2	NA	0	NA	0	0	0	1
5	10002	2	0	NA	0	0	2	0	0	0	0	0
6	10002	3	0	1	1	1	1	1	0	0	0	0

```
> print(trio.imp$trio[1:6,])
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
1	10001	1	0	0	1	1	1	1	0	0	0	0
2	10001	2	0	2	0	0	2	0	0	0	0	0
3	10001	3	0	1	1	1	1	1	0	0	0	0
4	10002	1	0	0	2	2	0	2	0	0	0	1
5	10002	2	0	1	0	0	2	0	0	0	0	0
6	10002	3	0	1	1	1	1	1	0	0	0	0

5 Detection of LD blocks

For the estimation of the haplotype structure that might be used in the R function `trio()`, this package also includes functions for the fast computation of the pairwise D' and r^2 values for hundreds or thousands of SNPs, and for the identification of LD blocks in these genotype data using a modified version of the algorithm proposed by Gabriel *et al.* (2002). For the latter, it is assumed that the SNPs are ordered by their position on the chromosomes.

These functions are not restricted to trio data, but can also be applied to population-based data. The only argument of these functions specifically included for trio data is `parentsOnly`. If set to `TRUE`, only the genotypes of the parents are used in the determination of the pairwise values of the LD measures and the estimation of the LD blocks. Furthermore, each parent is only considered once so that parents with more than one offspring do not bias the estimations. If trio data is used as input, the functions assume that the matrix containing the SNP data is in genotype format.

Here, we consider a simulated matrix `LDdata` from a population-based study. Thus, all subjects are assumed to be unrelated. This matrix contains simulated genotype data for 10 LD blocks each consisting of 5 SNPs each typed on 500 subjects. The pairwise D' and r^2 values for the SNPs in this matrix can be computed by

```
> data(trio.data)
> ld.out <- getLD(LDdata, asMatrix=TRUE)
```

where by the default these values are stored in vectors to save memory. If `asMatrix` is set to `TRUE`, the values will be stored in matrices. The pairwise LD values for the first 10 SNPs (rounded to the second digit) can be displayed by

```
> round(ld.out$Dprime[1:10,1:10], 2)
```

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
S1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S2	0.99	NA	NA	NA	NA	NA	NA	NA	NA	NA
S3	0.98	1.00	NA	NA	NA	NA	NA	NA	NA	NA
S4	0.98	0.99	1.00	NA	NA	NA	NA	NA	NA	NA
S5	0.97	0.98	0.99	1.00	NA	NA	NA	NA	NA	NA
S6	0.09	0.06	0.05	0.05	0.04	NA	NA	NA	NA	NA
S7	0.11	0.09	0.08	0.08	0.07	0.99	NA	NA	NA	NA
S8	0.13	0.11	0.10	0.10	0.09	0.99	1.00	NA	NA	NA
S9	0.14	0.11	0.10	0.11	0.10	0.99	1.00	1.00	NA	NA
S10	0.16	0.13	0.11	0.12	0.11	0.97	0.98	0.98	1	NA

```
> round(ld.out$rSquare[1:10,1:10], 2)
```

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
S1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S2	0.97	NA	NA	NA	NA	NA	NA	NA	NA	NA
S3	0.94	0.97	NA	NA	NA	NA	NA	NA	NA	NA

S4	0.93	0.96	1.00	NA	NA	NA	NA	NA	NA	NA
S5	0.91	0.94	0.98	0.98	NA	NA	NA	NA	NA	NA
S6	0.00	0.00	0.00	0.00	0	NA	NA	NA	NA	NA
S7	0.00	0.00	0.00	0.00	0	0.97	NA	NA	NA	NA
S8	0.00	0.00	0.00	0.00	0	0.95	0.98	NA	NA	NA
S9	0.00	0.00	0.00	0.00	0	0.93	0.96	0.98	NA	NA
S10	0.00	0.00	0.00	0.00	0	0.91	0.94	0.96	0.98	NA

and the pairwise LD plot for all SNPs can be generated by

```
> plot(ld.out)
```

(see Figure 1). This figure shows the r^2 -values. The D' values can be plotted by

```
> plot(ld.out, "Dprime")
```

(not shown).

The LD blocks in genotype data can be identified using the modified algorithm of Gabriel *et al.* (2002) by calling

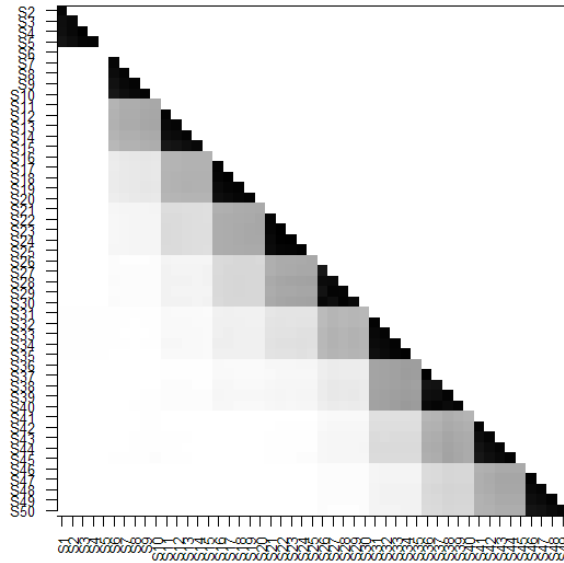


Figure 1: Pairwise r^2 values for the SNPs from LDdata.

```

> blocks <- findLDblocks(LDdata)
> blocks

Found 10 LD blocks containing between 5 and 5 SNPs.
0 of the 50 SNPs do not belong to a LD block.

Used Parameter:
Strong LD:      C_L >= 0.7 and C_U >= 0.98
Recombination: C_U < 0.9
(C_L and C_U are the lower and upper bound of
the 90%-confidence intervals for D')
LD blocks: Ratio >= 9

```

Alternatively, the output of `getLD()` can be used when `addVarN` has been set to `TRUE` in `getLD()` to store additional information on the pairwise LD values.

```

> ld.out2 <- getLD(LDdata, addVarN=TRUE)
> blocks2 <- findLDblocks(ld.out2)
> blocks2

Found 10 LD blocks containing between 5 and 5 SNPs.
0 of the 50 SNPs do not belong to a LD block.

Used Parameter:
Strong LD:      C_L >= 0.7 and C_U >= 0.98
Recombination: C_U < 0.9
(C_L and C_U are the lower and upper bound of
the 90%-confidence intervals for D')
LD blocks: Ratio >= 9

```

The blocks can also be plotted by

```

> plot(blocks)

```

(see Figure 2). In this figure, the borders of the LD blocks are marked by red lines. By default, the three categories used by the algorithm of Gabriel *et al.* (2002) to define the LD blocks are displayed. Since this algorithm is based on the D' values, it is also possible to show these values in the LD (block) plot.

```

> plot(blocks, "Dprime")

```

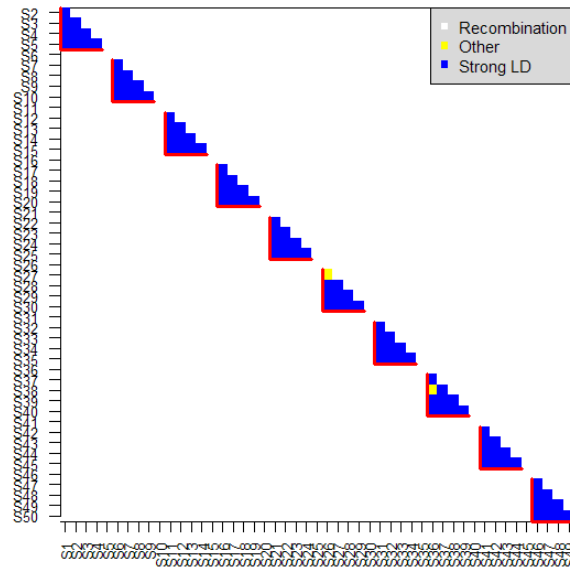



Figure 2: LD blocks as found by the modified algorithm of Gabriel *et al.* (2002). The borders of the LD blocks are marked by red lines. The color for the LD between each pair of SNPs is defined by the three categories used by Gabriel *et al.* (2002) to define the LD blocks.

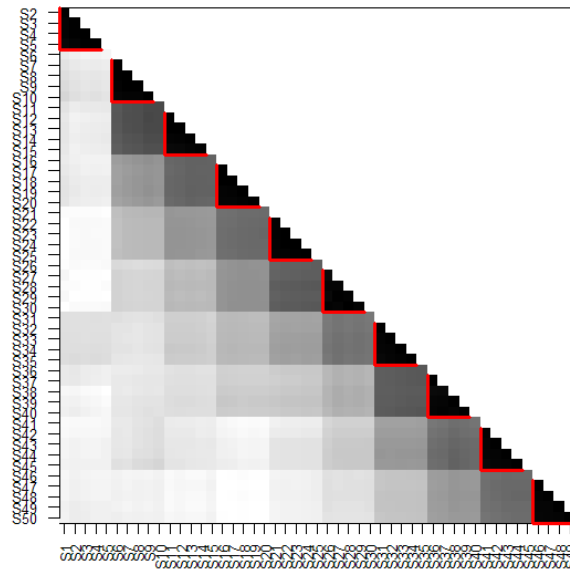


Figure 3: LD blocks as found by the modified algorithm of Gabriel *et al.* (2002). The borders of the LD blocks are marked by red lines. The darker the field for each pair of SNPs, the larger is the D' value for the corresponding SNP pair.

(see Figure 3).

As mentioned in Section 4, the haplotype structure required by `trio()` can be obtained by

```
> hap <- as.vector(table(blocks$blocks))
> hap

[1] 5 5 5 5 5 5 5 5 5 5
```

6 Simulation

The function `trio.sim()` simulates case-parents trio data when the disease risk of children is specified by (possibly higher-order) SNP interactions. The mating tables and the respective sampling probabilities depend on the haplotype frequencies (or SNP minor allele frequencies when the SNP does not belong to a block). This information is specified in the `freq` argument of the function `trio.sim()`. The probability of disease is assumed to be described by the logistic term $\text{logit}(p) = \alpha + \beta \times \text{Interaction}$, where $\alpha = \text{logit}(\text{prev}) = \log(\frac{\text{prev}}{1-\text{prev}})$ and $\beta = \log(\text{OR})$. The arguments `interaction`, `prev` and `OR`, are specified in the function `trio.sim()`. Generating the mating tables and the respective sampling probabilities, in particular for higher order interactions, can be very CPU and memory intensive. We show how this information, once it has been generated, can be used for future simulations, and thus, speed up the simulations dramatically.

6.1 A basic example

We use the built-in object `simuBkMap` in a basic example to show how to simulate case-parent trios when the disease risk depends on (possibly higher order) SNP interactions. This file contains haplotype frequency information on 15 blocks with a total of 45 loci. In this example, we specify that the children with two variant alleles on SNP1 and two variant alleles on SNP5 have a higher disease risk. We assume that `prev = 0.001` and

OR = 2 in the logistic model specifying disease risk, and simulate a single replicate of 20 trios total.

```
> data(trio.data)
> str(simuBkMap)

'data.frame':      66 obs. of  3 variables:
 $ key : Factor w/ 15 levels "10-1","10-10",...: 1 1 1 8 8 8 8 9 9 9 ...
 $ hap : int   11 21 22 121 122 111 222 21 22 12 ...
 $ freq: num   0.099 0.228 0.673 0.006 0.026 0.1 0.867 0.079 0.441 0.48 ...

> simuBkMap[1:7,]

   key hap  freq
1 10-1  11 0.099
2 10-1  21 0.228
3 10-1  22 0.673
4 10-2 121 0.006
5 10-2 122 0.026
6 10-2 111 0.100
7 10-2 222 0.867

> sim <- trio.sim(freq=simuBkMap, interaction="1R and 5R", prev=.001, OR=2,
+ n=20, rep=1)
> str(sim)

List of 1
 $ : num [1:60, 1:47] 1 1 1 2 2 2 3 3 3 4 ...
 ..- attr(*, "dimnames")=List of 2
 .. ..$ : NULL
 .. ..$ : chr [1:47] "famid" "pid" "snp1" "snp2" ...

> sim[[1]][1:6, 1:12]

      famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
[1,]     1   1   1   1   1   1   1   2   2   1   0   2
[2,]     1   2   2   1   2   2   2   2   2   2   2   0
[3,]     1   3   2   2   1   1   1   2   2   2   1   1
[4,]     2   1   2   1   2   2   2   2   1   2   1   1
[5,]     2   2   2   2   2   2   2   2   2   2   2   1
[6,]     2   3   2   1   2   2   2   2   1   2   2   0
```

6.2 Using estimated haplotype frequencies

In this example we estimate the haplotype frequencies in the built-in data set `trio.gen1`, which contains genotypes for 10 SNPs in 100 trios. These estimated frequencies are then

used to simulate 20 trios for the above specified disease risk model.

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.gen1, is.linkage=FALSE)
> trio.impu <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=TRUE)
> str(trio.impu, max=2)
```

List of 3

```
$ bin : num [1:400, 1:21] 3 0 0 0 3 0 0 0 3 0 ...
..- attr(*, "dimnames")=List of 2
$ miss: NULL
$ freq:'data.frame':      19 obs. of  3 variables:
..$ key :Class 'AsIs' chr [1:19] "ch-1" "ch-1" "ch-h2" "ch-h2" ...
..$ hap : num [1:19] 1 2 1111 1112 1121 ...
..$ freq: num [1:19] 0.9425 0.0575 0.325 0.2225 0.0075 ...
```

```
> trio.impu$freq[1:7,]
```

	key	hap	freq
1	ch-1	1	9.425000e-01
2	ch-1	2	5.750000e-02
3	ch-h2	1111	3.250000e-01
4	ch-h2	1112	2.225000e-01
5	ch-h2	1121	7.500000e-03
6	ch-h2	1221	3.350000e-01
7	ch-h2	1222	3.701724e-09

```
> sim <- trio.sim(freq=trio.impu$freq, interaction="1R and 5R", prev=.001, OR=2,
+ n=20, rep=1)
> str(sim)
```

List of 1

```
$ : num [1:60, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
..- attr(*, "dimnames")=List of 2
.. ..$ : NULL
.. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...
```

```
> sim[[1]][1:6, ]
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
[1,]	1	1	1	0	0	0	0	1	0	0	0	1
[2,]	1	2	0	0	1	1	1	1	0	0	0	0
[3,]	1	3	1	0	1	1	0	0	0	0	0	1
[4,]	2	1	0	0	1	1	0	1	0	0	0	2
[5,]	2	2	0	0	1	1	1	2	0	0	0	1
[6,]	2	3	0	0	0	0	1	2	0	0	0	2

As before, the object containing the haplotype frequency information can also be generated from external haplotype frequencies and SNP minor allele frequencies. In the following

example we specify the haplotype frequencies, and generate two replicates of ten trios each.

```
> data(trio.data)
> sim <- trio.sim(freq=freq.hap, interaction="1R or 4D", prev=.001, OR=2,
+ n=10, rep=2)
> str(sim)

List of 2
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
   ..- attr(*, "dimnames")=List of 2
   .. ..$ : NULL
   .. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
   ..- attr(*, "dimnames")=List of 2
   .. ..$ : NULL
   .. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...

> sim[[1]][1:6,]

      famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
[1,]      1   1    1    0    0    0    0    1    0    0    0    0
[2,]      1   2    0    0    1    1    1    1    0    0    0    0
[3,]      1   3    1    0    1    1    0    1    0    0    0    0
[4,]      2   1    0    0    1    1    0    1    0    0    0    0
[5,]      2   2    0    0    1    1    1    1    0    0    0    1
[6,]      2   3    0    0    1    1    0    1    0    0    0    1
```

6.3 Using step-stones

Generating the mating tables and the respective sampling probabilities necessary to simulate case-parent trios can be very time consuming for interaction models involving three or more SNPs. In simulation studies, many replicates of similar data are usually required, and generating these sampling probabilities in each instance would be a large and avoidable computational burden (CPU and memory). The sampling probabilities depend foremost on the interaction term and the underlying haplotype frequencies, and as long as these remain constant in the simulation study, the mating table information and the sampling probabilities can be “recycled.” This is done by storing the relevant information (denoted as “step-stone”) as a binary R file in the working directory, and loading the binary file again

in future simulations, speeding up the simulation process dramatically. It is even possible to change the parameters `prev` and `OR` in these additional simulations, as the sampling probabilities can be adjusted accordingly.

In the following example, we first simulate case-parent trios using a three-SNP interaction risk model, and save the step-stone object. We then simulate additional trios with a different parameter `OR`, using the previously generated information.

```
> data(trio.data)
> sim <- trio.sim(freq=freq.hap, interaction="1R or (6R and 10D)", prev=.001,
+ OR=2, n=10, rep=1)
> str(sim)
```

```
List of 1
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
  ..- attr(*, "dimnames")=List of 2
    .. ..$ : NULL
    .. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...
```

```
> sim[[1]][1:6,]
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
[1,]	1	1	0	0	2	2	0	0	0	1	1	0
[2,]	1	2	1	0	1	1	0	2	0	1	1	0
[3,]	1	3	1	0	1	1	0	1	0	2	2	0
[4,]	2	1	1	0	0	0	0	2	0	0	0	0
[5,]	2	2	0	0	0	0	2	1	1	0	0	0
[6,]	2	3	1	0	0	0	1	2	1	0	0	0

```
> sim <- trio.sim(freq=freq.hap, interaction="1R or (6R and 10D)", prev=.001,
+ OR=3, n=10, rep=1, step.save="step3way")
> str(sim, max=1)
```

```
List of 1
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
  ..- attr(*, "dimnames")=List of 2
```

```
> sim[[1]][1:6,]
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
[1,]	1	1	0	0	2	2	0	2	0	0	0	0
[2,]	1	2	0	0	0	0	0	1	0	0	0	1
[3,]	1	3	0	0	1	1	0	2	0	0	0	1
[4,]	2	1	0	0	2	2	0	1	0	1	1	0
[5,]	2	2	1	0	1	1	0	1	0	0	0	0
[6,]	2	3	0	0	2	2	0	2	0	0	0	0

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