Using the SIB-PAIR program to Analyse genetic data:

A Gentle Introduction

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Using SIB-PAIR

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by

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INTRODUCTION

Sib−pair is a computer program for the manipulation and statistical analysis of genetic datasets. It implements a simple interpreted language in which the user writes commands. These can be entered interactively, or submitted as a batch from a text file in the usual way.

I have developed the program over a number of years in the open software way of "scratching an itch". That is, Sib−pair carries out practical procedures which I have required in the day−to−day handling of genetic data, and were not available using other computer programs and/or were interesting as a research or educational question.

These include:

- Imputation of missing genotypes or phenotypes.
- Simulation of genotype or phenotype data.
- Estimation of allele frequencies in codominant genetic systems.
- Testing of genetic equilibria: Hardy−Weinberg and linkage −− exact or maximum−likelihood loglinear models.
- Simple and complex segregation analysis of a binary trait.
- Estimation of familial correlations and sibship variances for a quantitative trait. Variance components analysis of quantitative and binary traits using a variety of likelihoods. Segregation analysis of a quantitative trait.
- Haseman−Elston sib−pair regression of a quantitative (or binary) trait using full and half−sib data, and variance components linkage analysis for normally distributed quantitative traits.
- Multiple versions of the transmission−disequilibrium test.
- Testing allelic association with a binary or quantitative trait −− Monte Carlo simulation of null distribution of simple tests, or now "measured genotype" familial analysis including combined association and linkage analysis.
- Single locus Affected Pedigree Method identity−by−state and identity−by−descent linkage analysis. This includes Wards [1993] extensions to include unaffected pedigree members.
- Writing out of locus and pedigree files in the formats used by the programs APM, Arlequin, ASPEX, CRIMAP, FISHER, GAS, GDA, Genehunter, LINKAGE, LOKI, MENDEL, MERLIN, PAP and SAGE.

There are also a variety of standard statistical procedures, such as multiple regression analysis, contingency table analysis, survival analysis, random number generators, quantiles for assorted statistical distributions.

Finally, there are a few utilities that give expectations for specified genetic models eg recurrence risks for a single major locus model.

In this document, I try to give a gentle introduction to using Sib−pair. The other sources of information about Sib−pair are the manual, the extended help pages for each command, and reading the Fortran 95 source code (it does have some comments!).

A very first session

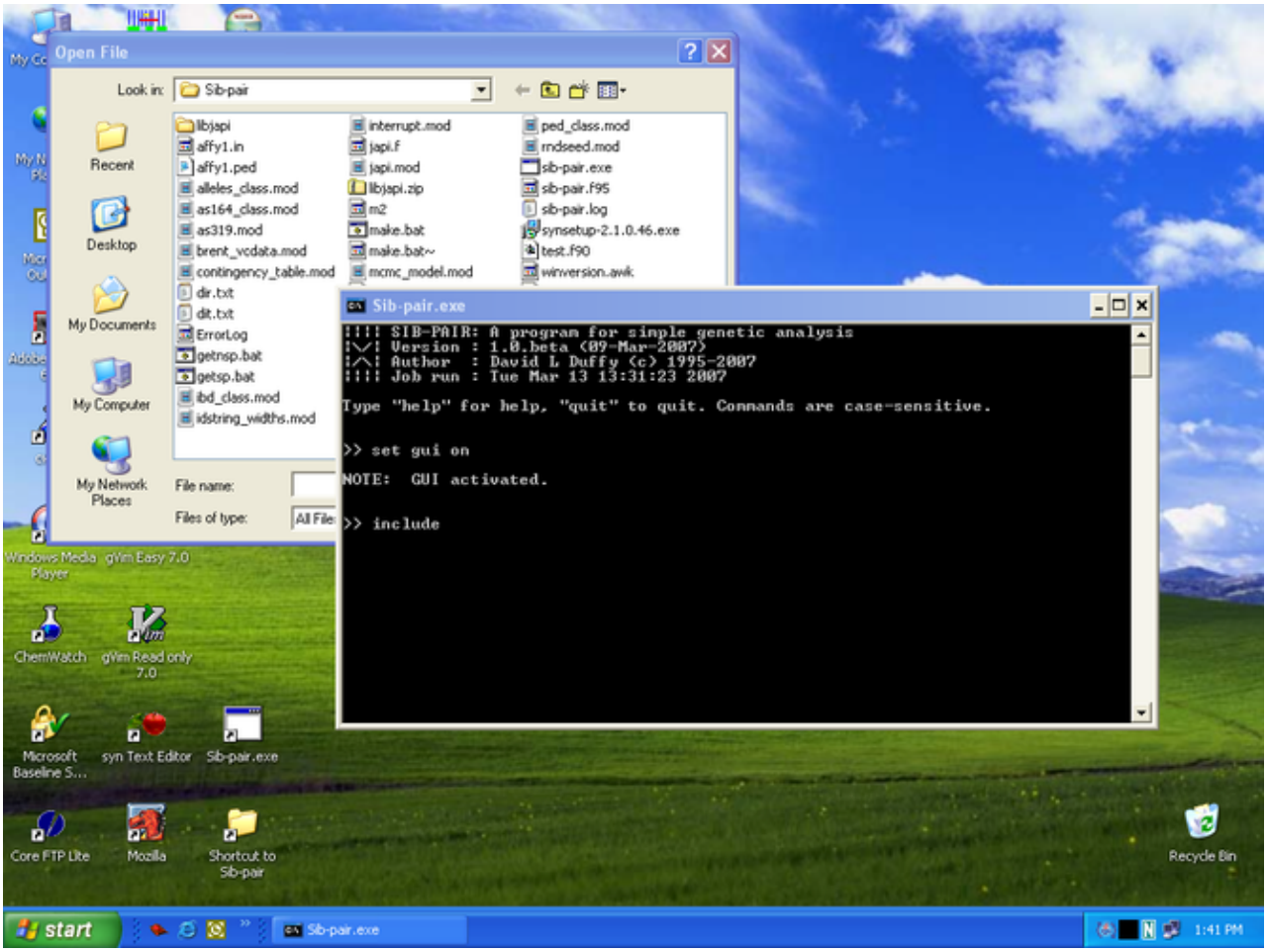
Sib−pair is not difficult to use. In a Windows environment, double−click the icon or open a DOS box in your working directory (folder) and call Sib−pair from the command line. The latter assures that Sib−pair will find your pedigree files and write output in the same directory. In a Unix environment, run Sib−pair from the command line.

```
davidD@moonboom:~$ sib-pair
|||| SIB-PAIR: A program for simple genetic analysis
|\/| Version : Version 1.00.beta (23-May-2007)
|/\| Author  : David L Duffy (c) 1995-2007
|||| Job run : Thu May 24 14:36:54 2007 (orpheus.qimr.edu.au)

Type "help" for help, "quit" to quit, "ctrl-C" to interrupt.


>>
```

You see much the same on Windows.



Now type in "help" on the command line (followed by <ENTER>):

```
>> help
Keywords can be shortened to the first 3 letters.
Help prints a brief description of a command or group of commands:

  help [ <search string> | All | Globals | Operators | Data | Analysis | Examples]

For full online help:

  $ your_favourite_HTML_browser sib−pair.html


Now try "help Examples"
```

Entering "help All" gives a long list of all the commands (see Appendix).

To list just the commands mentioning "merlin":

```
>> help merlin
rea loc mer <fil> {read Merlin locus file}
wri mer [dum] <fil> {write Merlin pedigree file}
wri loc mer <fil> {write Merlin locus file}
wri map mer <fil> {write Merlin map file}
```

The latter three commands can produce the files needed to run a multipoint linkage analysis in MERLIN.

Here is the list of commands mentioning "risk". Then we will try the "sml" (short for single major locus) command.

```
>> help risk
sml <pA> <penAA> <penAB> <penBB> {recurrence risks}
grr <prev> <pA> <GRR> [<add|dom|rec>] {recurrence risks}
hrr <tra> [<c_op> <thr>] {haplotype relative risk}

>> sml 0.17 0.21 0.08 0.03

------------------------------------------------
Single Major Locus Recurrence Risk Calculation
------------------------------------------------

 Frequency(A): 0.170000; Pen(AA): 0.210; Pen(AB): 0.080; Pen(BB): 0.030
 Trait Prev  : 0.049312; Pop AR:  39.2%; Var(Add): 0.001141; Var(Dom): 0.000127

 Measure      MZ Twin       Sib−Sib        Par−Off      Second
----------   ----------    ----------     ---------    ----------
 Rec risk         0.075         0.062         0.061         0.055
 Rel risk         1.564         1.264         1.250         1.124
 Odds rat         1.610         1.281         1.266         1.131
 PRR              1.522         1.248         1.235         1.117
 ibd|A−A          1.000         0.552         0.500         0.276
 ibd|A−U          1.000         0.497         0.500         0.248

 Freq of A if Affected: 0.351983 (0.123,0.458,0.419)
 Freq of A if Unaffctd: 0.160561 (0.024,0.273,0.703)

 Mating       Proportion    Risk to offspring
----------   -----------   ------------------
UnA x UnA         0.904         0.048
Aff x UnA         0.094         0.060
Aff x Aff         0.002         0.075
```

The "sml" results show that a gene with the given allele frequency and penetrances (APOE*E4 and Alzheimer's disease by age 75) give rise to a recurrence risk of 6.2% in siblings of cases, a 1.3−fold increase in risk compared to the baseline population risk of 4.9%. The frequency of the risk allele (here APOE*4) is doubled in cases when compared to controls, and *IBD* sharing (Identity−by−Descent) in affected sib pairs is expected to be 55%.

Simultaneously pressing the combination of the "Ctrl" and "c" keys interrupts whatever calculation Sib−pair is currently doing and returns you back to the Sib−pair command prompt. Usually Sib−pair will first try to complete a part of the calculation and give a result, so there may be a small delay. Pressing "Ctrl−C" multiple times causes the Sib−pair to stop completely, and returns you to the operating system.

Two last things:

```
>> 1+1 ; 2+2
=>  2.
=>  4.

>> quit

This job took  28.1 minutes
```

A genetic analysis from scratch

In this case, I will go through the analysis of some pedigree data from beginning to end. I won't describe the commands in too much detail, as they will be covered later.

Usually, I have:

1. A description of the pedigree, such as a pedigree diagram
2. Trait information about individuals in the pedigree, such as disease status, or age or body mass index.
3. Genotype information about individuals in the pedigree, which will usually be for codominant loci, but can be mitochondrial or Y−chromosomal.

Here is a diagram of a pedigree described in a paper by Lawler and Sandler (1954) where members are affected by the condition familial elliptocytosis.

The diagram in Morton (1956):

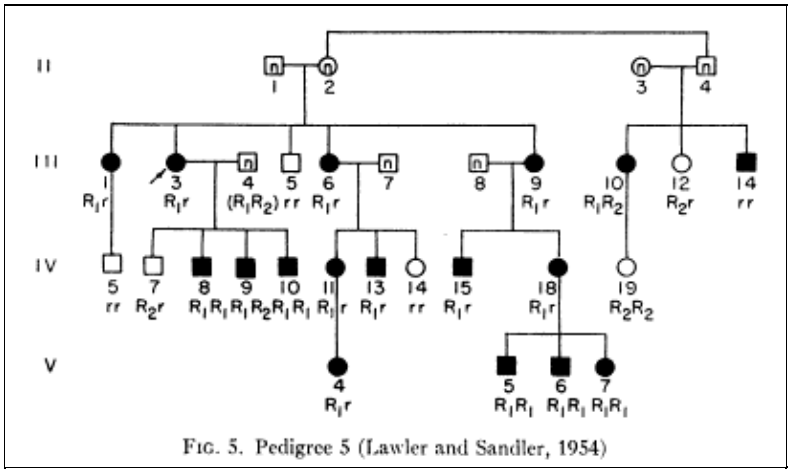
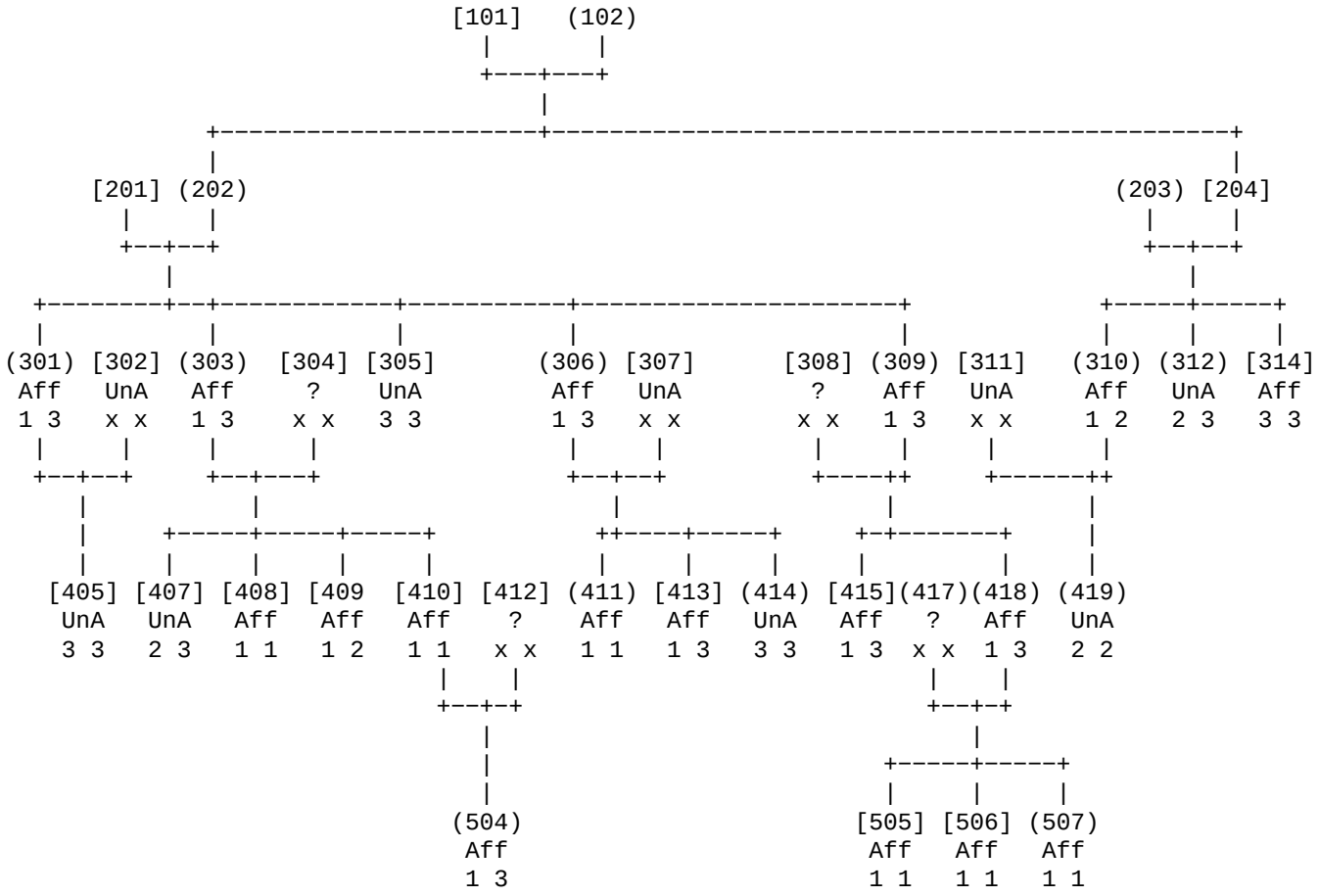


FIG. 5. Pedigree 5 (Lawler and Sandler, 1954)

And here is the same diagram amended to include some additional necessary family members, and recoding the alleles to 1, 2, and 3 (Sib−pair only allows **single letter** or **numerical** allele codes):



Note every pedigree member has been given a unique identifier. In the later generations, there is information as to whether individuals are affected or unaffected (by elliptocytosis), and their genotype at the Rhesus blood group locus. Each genotype is given as the two alleles.

From this, I will make a single **plain text** file that contains all this information in the form that Sib−pair reads. The pedigree is written as one line per pedigree member. Each line contains the same number of items separated by spaces. The columns are:

Column	Name
1	Pedigree name
2	Individual identifier
3	Father's identifier
4	Mother's identifier
5	Sex (**m** or **f**)
6	Affected or unaffected by elliptocytosis (**y** or **n**)
7	First allele of Rhesus genotype (**1**, **2** or **3**)
8	Second allele of Rhesus genotype (**1**, **2** or **3**)

Because columns are "free format" white−space separated, if a value is missing, there must be a placeholder to identify the column (Sib−pair uses an "**x**" for this purpose).

I use the gvim text editor, but you can use NotePad or any other editor. We open a new document. Now we go through the pedigree diagram person by person, adding one line to our document for each person. The first record (for person 101) will be:

```
ped5 101 x x m x x x
```

The parent identifiers are set to "x" because they are not given. Furthermore the trait status and genotype are also unknown. Where sex is not given in the diagram, we will assign them so that the marriages in the drawing are consistent.

The first "nonfounder" (that is a person with parents included in the diagram) is written as:

```
ped5 202 101 102 f x x x
```

Skipping forward, the last pedigree record will be:

```
ped5 507 417 418 f y 1 1
```

The pedigree columns now need to be identified in such a fashion that Sib−pair can recognize them. At the start of the file, I first prepend a comment, so I know what this file does. Comment lines are ignored by Sib−pair and are prefixed by a "#" at the start of the line:

```
#
# Elliptocytosis pedigree 5 from Lawler & Sandler 1954, Morton 1956
# Zmax of 3.31 at c=0.05
#
```

Following this, we declare the columns. The first five columns are standard and so don't need to be named. The sixth column is disease state, so the first line of the declarations (using the "`set locus`" command) is:

```
set locus ellipto affection
```

The seventh and eight columns represent the alleles of the genotype, so the second declaration line is:

```
set locus rhesus marker
```

To show where the pedigree data starts, we add a line:

```
read pedigree inline
```

The end of the pedigree data is marked by ";;;;" on its own line. After this we add the "run" command, which tells Sib−pair to process the pedigree. We save the completed script to a file "*ellipto5ex.in*". Its contents looks like this.

So we can start up Sib−pair, and "include" the contents of this file into our session:

```
>> include ellipto5ex.in
```

If you got the message

```
>> include ellipto5ex.in

ERROR:  Unable to open "ellipto5ex.in".
```

Then you are not in the correct directory (folder). The "pwd" command in Sib−pair shows you which directory you are in, and can be used to change directory as well. Alternatively, you can try again, using just

```
>> include
```

which will give you some type of file chooser menu. This will allow you to navigate to the correct directory and include the file. You should now get:

```
Reading commands from "ellipto5ex.in".
!
! Elliptocytosis pedigree 5 from Lawler & Sandler 1954, Morton 1956
! Zmax of 3.31 at c=0.05
!
Pedigree file        = inline.ped
Number of loci       =     2

Locus           Type Position
--------------- ---- ----------------
ellipto          a         6
rhesus           m         7--      8

Number of marker loci=       1
Bonferroni corr. 5%  =    0.050000
Bonferroni corr. 1%  =    0.010000
Bonferroni corr. 0.1%=    0.001000

 Max record length is  25  characters
 Screened  1  pedigrees,  36  records (0.00 s).
 Read in   1  pedigrees,  36  individuals (0.01 s).
 Dataset occupies 0.002 Mb.

No sex−informative markers.
Nuclear family error checking.

Nuclear family error checking completed.

Number of data problems    =     0
Starting values for missing genotypes generated.
```

```
Total number of pedigrees  =       1
Number with only 1 member  =       0
Total number of sibships   =      10
Total number of subjects   =      36
Total subjects genotyped   =      23 (63.9%)
Total number of genotypes  =      23
Largest pedigree (members) =      36

Mean size of pedigrees     =      36.0

Closing include file "ellipto5ex.in".

>>
```

So I have successfully read in the pedigree data, and there are no messages about errors of various types. The "describe" command tells me about the phenotype (*ellipto*) and genotypes (*rhesus*):

```
>> describe

------------------------------------------------
Segregation ratios for trait "ellipto   "
------------------------------------------------

Total sample   All       Fndrs     Nonfndrs
-----------------------------------------
   Aff/Tot    17/  23    0/   0   17/  23
   Prop Aff     0.739     0.000     0.739
   Missing         13        11         2

Mating Type     UxU       UxA       AxA
-----------------------------------------
   Matings          0         0         0
   Aff/Tot     0/   0    0/   0    0/   0
   Prop Aff     0.000     0.000     0.000

Relative pair  RecRisk   Aff−Aff   Aff−UnA
-----------------------------------------
   Marital      0.000         0         0
   Gparent      1.000         4         0
   Halfsib      0.000         0         0
   Par−Off      0.846        11         4
   Fullsib      0.732        15        11

------------------------------------------------
Allele frequencies for locus "rhesus"
------------------------------------------------
   Allele  Frequency   Count  Histogram
       1     0.4783       22  **********
       2     0.1304        6  ***
       3     0.3913       18  ********

Number of alleles    =    3
Heterozygosity (Hu)  =    0.6145
Poly. Inf. Content   =    0.5181
4 Neff mu (SSMM)     =    2.44529900
Number persons typed =     23 ( 63.9%)
```

I can see that 17 pedigree members are affected by the condition, and that there are 23 individuals genotyped. The "hwe" command doesn't suggest marker problems.

```
>> hwe
```

```
------------------------------------------------
Hardy−Weinberg equilibrium for marker loci
------------------------------------------------

Marker         Typed  Genos  Chi−square Asy P  Emp P  Iters
-------------- ------ ------ ---------- ------ ------ ------
rhesus             23      6        1.2 0.7496 0.8696     23 HWE .
```

Is there genetic linkage between familial elliptocytosis and Rhesus blood group? Sib−pair offers a nonparametric identity−by−descent test of linkage ("apm"):

```
>> apm ellipto ibd

------------------------------------------------
APM for trait "   ellipto" v. all markers
------------------------------------------------

NOTE:  Identity−by−descent based statistic used.


Marker         NFams  NAff   Z−value    Asy P  Emp P  Iters
-------------- ------ ------ ---------- ------ ------ ------
    rhesus          1     17        1.7 0.0443 0.0700    200 APM−IBD +
    rhesus          1     17        4.1 0.0000 0.0050    200 GPM−IBD ***
```

There are results from two tests here, one using affected pedigree members only (APM), and the other including information from unaffecteds as well (GPM). The latter is more impressive, with a Z−score of 4.1 being the equivalent of a lod score of

```
>> 4.1^2 / (2*log(10))
=>  3.650245120396831
```

This score seems a bit high perhaps (I know that the parametric lod score is only 3.3). Like many Sib−pair tests, this test is simulation based, so it is worthwhile increasing the number of simulations used to refine the estimates:

```
>> set iterations 10000
>> apm ellipto ibd

------------------------------------------------
APM for trait "   ellipto" v. all markers
------------------------------------------------

NOTE:  Identity−by−descent based statistic used.

Marker         NFams  NAff   Z−value    Asy P  Emp P  Iters
-------------- ------ ------ ---------- ------ ------ ------
    rhesus          1     17        1.4 0.0757 0.0852  10000 APM−IBD +
    rhesus          1     17        4.3 0.0000 0.0024  10000 GPM−IBD ***
```

So, elliptocytosis seems to be linked to this marker, but the empirical P−value is equivalent to a lod of 2. We can look at some other linkage tests, including the original Penrose sib−pair linkage test:

```
>> pen ellipto rhesus

------------------------------------------------------------
Penrose Sib Pair Linkage Analysis for "ellipto" v. "rhesus"
```

```
_____________________________________________________________

                     rhesus
ellipto    Concordant  Discordant
Concordant         11           4
Discordant          0          11

No. of sib pairs   =    26
No. of sibships    =     6

    No. complete observations =    26
    LR contingency chi−square =  18.03
           Degrees of freedom =   1
           Asymptotic P−value =   0.0000
              Empiric P−value =   0.0001 ( 2600000 MCMC iterations)
             Trend chi−square =  13.44   (P=0.0002)
                    Agreement =   0.846  (   22/   26)
                Cohen's Kappa =   0.6994
```

We can also look at this using the assoc test:

```
>> set plevel 1
>> assoc ellipto

------------------------------------------------
Allelic association testing for trait "ellipto"
------------------------------------------------

  ---- Association Analysis for "rhesus    "------
    Allele  Affected    Unaffected    Total    Dev
  ------------------------------------------------
     1       22 (.647)      0 (.000)      22    8.1
     2        2 (.059)      4 (.333)       6    0.4
     3       10 (.294)      8 (.667)      18    2.9
  ------------------------------------------------
   Total     34            12             23

       No. trait(+) marker(−) =     0
       No. trait(+) marker(+) =    23
                 Fis, Fit, Fst =   0.0818   0.4125   0.3602
   Contingency Pearson chi−sq =  16.0
   Nominal degrees of freedom =   2
              Nominal P−value =   0.0003
      Equalled or exceeded by =   1/10001 simulated values (0.0001)
 Mean (Var) simulated chi−sqs =   1.7 (   2.7)

 -------- Combined transmission test for "    rhesus" --------
   Allele   Affected   Unaffected   Total   E(Aff)    Z    P
 ------------------------------------------------------------
     1       13 (.59)      0 (.00)     13    7.9    2.8 0.0053
     2        2 (.09)      2 (.25)      4    2.4   −0.4 0.6653
     3        7 (.32)      6 (.75)     13   11.7   −2.6 0.0094
 ------------------------------------------------------------
   Total     22            8           30

                    marker(−) =     1
       No. trait(+) marker(+) =    15
          No. useful sibships =     4
 Global association statistic =   2.5
           Degrees of freedom =   2
      Equalled or exceeded by =  20/ 1019 simulated values (0.0196)
 Mean (Var) simulated chi−sqs =   0.5 (   0.3)
```

Because the assoc empirical P−value is generated by gene dropping, in a single pedigree like this it gives a valid test of cosegregation of alleles at the Rhesus locus with elliptocytosis. The "1" allele was never seen in an unaffected individual. The FBAT (combined transmission test) confirms children with elliptocytosis received the "1" allele more often than expected.

Finally, I'll use Sib−pair to output the files that Superlink requires for a parametric linkage analysis. I'll model elliptocytosis as due to a rare fully penetrant dominant locus:

```
>> set sml 0.0001 1 1 0
SML model: P(A)=0.000100 Pen(AA)=1.000 Pen(AB)=1.000 Pen(BB)=0.000

>> write locus linkage el.loc

Writing LINKAGE type locus file: el.loc

>> write ppd el.ppd

Writing post−Makeped Linkage style pedigree file: el.ppd
```

I'll then change a couple of parameters (the starting recombination distance between *ellipto* and *rhesus*, and the evaluation step size) in the locus file "*el.loc*" using my favourite text editor. Then I'll run Superlink (for this to work you must have already installed Superlink into your directory path):

```
>> $ gvim el.loc
>> $ superlink el.loc el.ppd

....
                −6.391669        −51.787565     3.265655
                −5.268026        −51.752765     3.280768
                −4.169080        −51.760457     3.277427
                −3.093770        −51.834167     3.245415
                −2.041100        −52.026618     3.161835
                −1.010135        −52.505211     2.953985
   rhesus
                0.000000         −56.912210     1.040050
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
  MAX           −5.268026        −51.752765     3.280768
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
```

The maximum lod is 3.28 at 5.3 cM distant from the marker. The Rh−linked elliptocytosis gene (EPB41) is now known to lie at 29.2 Mbp from the 1p telomere (1p33−p32), and the Rhesus complex of genes at about 25.5 Mbp. They are separated by approximately 3.9 cM on the Rutgers linkage map.

We can actually fit the equivalent model in Sib−pair by creating a marker to represent elliptocytosis. and using the lod command.

```
>> set locus traitlocus marker
>> if (ellipto) then traitlocus="1/2"
>> if (not ellipto) then traitlocus="1/1"
>> set freq traitlocus 0.9999 0.0001

NOTE:  The marker "traitlocus" has prespecified allele frequencies:
       1=0.9999 2=0.0001

>> lod traitlocus rhesus

------------------------------------
Two−point lod score linkage analysis
------------------------------------

NOTE:  Population allele frequencies for "traitlocus" are prespecified as:
       1=0.9999 2=0.0001

"traitlocus" (2 alleles) v. "rhesus" (3 alleles).

 LogLikelihood   LOD       Theta
--------------  ---------  -------
    −59.3092        0.000   0.5000
    −56.2217        1.341   0.0001
    −52.5057        2.955   0.0100
    −51.9104        3.213   0.0250
    −51.7533        3.282   0.0500
    −51.8939        3.220   0.0750
    −52.1640        3.103   0.1000
    −52.9107        2.779   0.1500
    −53.8264        2.381   0.2000
    −55.9736        1.449   0.3000
    −58.2181        0.474   0.4000

>> quit

This job took   8.8 minutes
```

LANGUAGE ELEMENTS

There are over two hundred different commands in Sib−pair now (see Appendix). The language has an irregular grammar (like all good computer languages), with a mixture of three sorts of statements:

- Analytic/manipulation **commands**
- Algebraic (infix) or logical **expressions**
- **Macros**

These act upon two types of data:

- Scalar constants
- Dataset (vector) variables

Data

A scalar constant is either a number or a genotype value. A genotype is made up of two alleles separated by a forward slash and enclosed in a set of double quotes. Each allele can take the values 1...999 or a single letter A...Z and a...z (noting that "x" is reserved as a **missing allele**).

```
>> 10
=>  10.
>> "a/b"
=>    a/b
```

There are a few named constants:

```
>> pi
=>  3.14159265359
>> y
=> 1.
>> n
=> 0.
>> x
=> MISS
>> .
=> MISS
```

A variable is a column or vector of values in the dataset, and is identified by a unique variable name. The dataset is a rectangular matrix of numbers or genotypes (stored in computer memory), each row representing data for an individual. A variable can be of five types:

`marker`	autosomal genotype
`xmarker`	X−chromosome genotype
`haploid`	Y or mitochondrial genotype
`affection`	dichotomous trait value
`quantitative`	continuous trait value

The name and column location of a variable is declared by using the `set locus` command. Usually the values for a variable will be read into Sib−pair from a file (see below). Each row of the dataset is associated with a pedigree and individual ID, IDs of parents of the individual, sex of the individual.

The "head" command prints out the first 10 values of each variable. One can get an equivalent result using the "print" command:

```
>> head

!
!               S                                              p
!               e A                                            r
!   Per Fat Mot x D   onset      age    D14S52  D14S43  D14S53  o
!
AM  101 x   x   m y   43.0000     x       x/x     x/x     x/x   n
AM  102 x   x   f n   77.0000   77.0000  83/87  183/183 151/151 n
AM  203 x   x   f n     x          x      83/93  171/181 151/157 n
AM  201 101 102 m y   41.0000     x       x/x     x/x     x/x   y
AM  202 101 102 m y   43.0000     x       x/x     x/x     x/x   n
AM  204 101 102 m n   63.0000   63.0000   x/x     x/x     x/x   n
AM  205 101 102 m y   46.0000     x      83/87  183/183 151/155 n
AM  206 101 102 m y   41.0000     x      83/87  183/183 151/155 n
AM  207 101 102 m n   52.0000   52.0000   x/x     x/x     x/x   n
AM  208 101 102 m y   41.0000     x      83/87  183/183 151/155 n

>> print index < 10

Print where "index < 10":

ped=AM id=101 fa=x mo=x sex=m AD=y onset=43.0000 age=x D14S52=x D14S43=x D14S53=x proband=n
ped=AM id=102 fa=x mo=x sex=f AD=n onset=77.0000 age=77.0000 D14S52=83/87 D14S43=183/183 D14S5
ped=AM id=203 fa=x mo=x sex=f AD=n onset=x age=x D14S52=83/93 D14S43=171/181 D14S53=151/157 pr
ped=AM id=201 fa=101 mo=102 sex=m AD=y onset=41.0000 age=x D14S52=x D14S43=x D14S53=x proband=
ped=AM id=202 fa=101 mo=102 sex=m AD=y onset=43.0000 age=x D14S52=x D14S43=x D14S53=x proband=
ped=AM id=204 fa=101 mo=102 sex=m AD=n onset=63.0000 age=63.0000 D14S52=x D14S43=x D14S53=x pr
ped=AM id=205 fa=101 mo=102 sex=m AD=y onset=46.0000 age=x D14S52=83/87 D14S43=183/183 D14S53=
ped=AM id=206 fa=101 mo=102 sex=m AD=y onset=41.0000 age=x D14S52=83/87 D14S43=183/183 D14S53=
ped=AM id=207 fa=101 mo=102 sex=m AD=n onset=52.0000 age=52.0000 D14S52=x D14S43=x D14S53=x pr
```

There are no vector constants. One way to specify new values for an existing variable is to use the "edit" command

```
>> edit Smith John Chol to 6.85
Changing Smith−−John at locus "Chol" from   5.5000 to   6.8500
```

The alternative is to read in new values from a file using the href="./Commands/update.html">update" command.

Quantitative traits can also be dates, taking the form of 8 digit integers (*YYYYmmdd*). These can be converted to and from Julian days (number of days since an epoch, such as 01−Jan−1970 − the usual computing epoch; 16−Nov−1858 − the "reduced" Julian day or 01−Jan−4713 BCE − standard Julian day).

```
>> date 19000101
Date: 19000101 =    −25567
>> date onset
Converting dates at "onset" from Gregorian to Julian (epoch="1970−01−01").
```

Commands

Entering a command in Sib−pair causes the program to apply an algorithm to data. This leads either to a manipulation of the variables in the dataset "in−place", or the printing of a result to the standard output.

One issues one command per statement. The statement will start with the command name, that can usually be shortened to the first three letters, followed by white−space separated positional parameters: either numerical constants, modifier keywords or variable names.

```
>> help Globals
>> chi 2 2
>> pchisq 3.84 1
>> set locus Chol quantitative
>> set locus age quantitative
>> read pedigree cholesterol.ped
>> run
>> varcom Chol ae covariates age
```

These commands respectively printed out the on−line help summary for all global commands, performed a contingency chi−square analysis of a two−by−two table to be entered from the keyboard, printed the P−value for a specified chi−square value and degree of freedom, defined the names of two variables in the dataset for analysis, declared the file to read the dataset from, read that dataset into memory ("`run`"), and fitted a biometrical genetic linear mixed model to the "Chol" variable.

Algebraic expressions

The algebraic operations (which include if/then type statements) involve a set of standard operations applied either to constants, or to the vector of values for a given variable in the dataset. They return a value of the same size. If the result is a dataset variable, this results in that variable being updated, otherwise a result is written to the standard output.

A new variable in a dataset can be created by a "`set locus`" declaration if a dataset has already been read into memory by the "`run`" command.

```
>> 10^2 + sqrt 7
=>   102.64575131106459
>> "a/b" + 1
=> b/c
>> set locus logChol quantitative
>> logChol = log(Chol+1)
>> set locus hichol affection
>> if (male and Chol > 7) then hichol=y else hichol=n
```

Note that because command names can be shortened, there can be a clash between commands and the name of a variable starting an algebraic expression. This can be avoided by enclosing the variable name in brackets, forcing it to be evaluated as (part of) an expression (or using the `let` command).

```
>> set loc var qua
>> (var) = 1
# or equivalently
>> (var = 1)
# or equivalently
>> let var = 1
```

A sequence of expressions can be evaluated together, speeding up execution and extending usefulness of the `if−then` construction.

```
>> (a = index) (b=a^2−2*a+1)
>> if (b<0) then (a = b) (b=1/b)
```

Algebraic expressions cannot be passed directly to commands, so

```
 >> var (log(Chol+1))
```

does **not** work. However, a number of analysis commands allow simple comparison operators eg

```
 >> tdt Chol > 6.5
>> ass Group odd
```

are legal.

Macros

Macros (by definition) can contain any type of the last two types of command. Macro *functions* take positional parameters like analytic commands do.

```
>> macro add
add> %1 + %2
add> ;;;;
>> add 2 2
=> 4
```

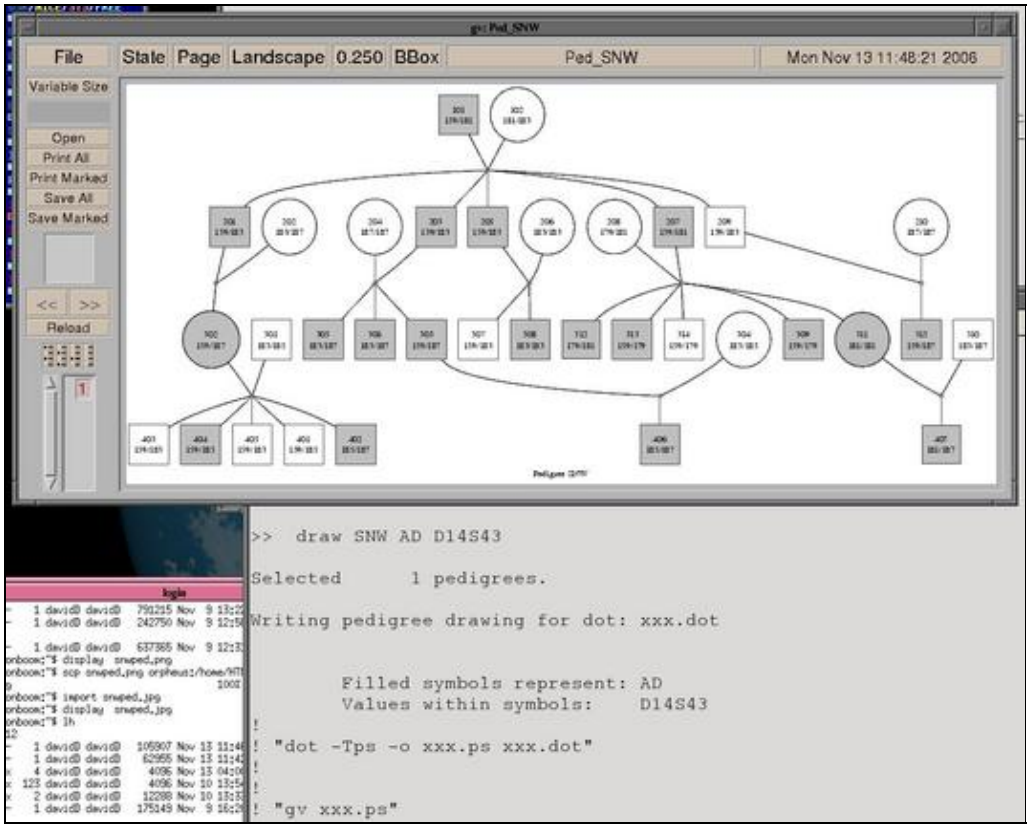
The macro declaration above consists of the `macro` keyword followed by the name of the new macro. The lines up until the final line (either ";;;;" or an empty line) are the body of the macro.

The macro parameters are represented by "%1", "%2", etc. When the macro is called, the "%1" in the body will be replaced by the first token (word, number) after the macro name, and so forth, and the new string will then be evaluated as either a command or algebraic expression.

In the example above, the newly defined `add` command adds together the first two arguments following the command name.

Macros can contain multiple commands and expressions.

```
# Comment lines are prefixed by "#" or "!"
# Draw a pedigree (need to have dot and gv):
# First set up macro
>> macro draw
draw> select pedigree %1
draw> write dot %%.dot %2 %3
draw> $ dot −Tps −o %%.ps %%.dot
draw> $ gv −−scale=−1 %%.ps
draw> $ rm %%.dot %%.ps
draw> unselect
draw>
# Call macro
>> draw SNW AD D14S43
```



This macro first selects a pedigree or pedigrees named by the first argument of the macro (`select` temporarily subsets the dataset, and allows wild−card searches). It then calls the "`write dot`" command to create a file in the dot graphical language to draw the pedigree as a marriage node diagram. The circle (female) or square (male) representing an individual in the drawing will be shaded if that person is affected at the trait (the second argument to the macro). The "`$`" command allows two other programs to be called by Sib−pair, *dot* and *gv* (ghostview). Finally, the intermediate files are deleted, and all the other pedigrees returned to the list of active pedigrees.

The intermediate file names were created using the %% variable, which contains a (random) character string uniquely identifying the current macro call.

Macros can also be used as *variables*. The value of the macro is set using the `macro` command, but the contents are accessed using %<macro_name>". A macro variable can appear anywhere within a normal command.

```
>> macro a=1
>> macro b=+
>> %a%b%a
=> 2.
>> macro a=D14S52 D14S43
>> tab %a
------------------------------------------------
Cross−tabulation of "D14S52" ... "D14S43"
------------------------------------------------
[...]
```

When a macro is evaluated as a macro variable, macro positional parameters (%1, %2 etc) within the macro body are not evaluated.

Macro iteration

This is another recent expansion of the language. Many Sib−pair commands automatically iterate over a class of eligible variables, for example the list of all active marker loci. In this case, the "keep" and "drop" commands can be used refine the selection.

For any command, a *list of tokens enclosed in braces* causes the command to be called repeatedly, substituting each member of the list into the command line at that location.

```
>> {1 2} + 1
=> 2.
=> 3.
>> tab { CHD carrier } ldl

------------------------------------------------
Cross−tabulation of "CHD" ... "ldl"
------------------------------------------------
                      ldl
CHD                1/1          1/2          2/2     Allele Freq  Exact HWE−P
           ------------------------------------------------------------------
     n          12 (.462)    11 (.423)     3 (.115)  0.6731  0.3269    1.0000
     y           0 (.000)     1 (1.00)     0 (.000)  0.5000  0.5000    1.0000
[...]
------------------------------------------------
Cross−tabulation of "carrier" ... "ldl"
------------------------------------------------
                      ldl
carrier            1/1          1/2          2/2     Allele Freq  Exact HWE−P
           ------------------------------------------------------------------
     n          24 (.774)     6 (.194)     1 (.032)  0.8710  0.1290    0.4027
     y           0 (.000)    13 (.867)     2 (.133)  0.4333  0.5667    0.0079

>> set loc a{1 2 3} aff; ls
a1* a2* a3*
 3  active traits;  0  active markers.
```

As can be seen in the last example, iteration is implemented in a macro fashion, and usually precedes evaluation of the other token. The exception to this is the handling of class summary tokens such as "$m" (all active autosomal markers) when they are the only value in the list. Multiple iteration and nested iteration are implemented.

```
>> 1{1 2} + {1 2}
=> 12.
=> 13.
=> 13.
=> 14.

>> 1{1 2 {3 4}}
=>  11.
=>  12.
=>  13.
=>  11.
=>  12.
=>  14.
```

DATASETS

Sib−pair can read in datasets in a variety of formats:

- Native format inline data
- Native format pedigree file
- Merlin format pedigree file
- Linkage format pedigree file
- Plink format .bed file

Inline data

The simplest way to get data into Sib−pair is as in−lined data in a script. The script below will read an example of such a script:

```
# declare four loci
set locus a affection
set locus b quantitative
set locus m1 marker 0.0 cM
set locus m2 marker 5.1 cM
# read the pedigree data
read pedigree inline
# ped.id ind.id fa.id mo.id sex a b m11 m12 m21 m22
ex1 1a x x m n x 1 3 1 2
ex1 1b x x f n x 1 2 3 4
ex1 2a 1a 1b m n 3.5 1 2 1 3
ex1 2b x x f n 1.1 2 2 2 3
ex1 3a 2a 2b m y 4.3 1 2 1 2
ex1 3b 2a 2b m n 2.0 2 2 2 3
ex1 3c 2a 2b f n 0.8 2 2 3 3
ex1 4a 3c 3d f y x 1 2 2 3
ex1 3d x x m n x x x x x
ex1 4b 3b 3e m y 4.7 1 2 3 4
ex1 4c 3b 3f m n 1.6 2 2 1 3
;;;;
# The four semicolons ends the in−line data
run
```

Datasets contain one record (newline character delimited) per individual. Records should be sorted into pedigrees (though they can be joined subsequently with the "`join`" command). Records take the format used by GAS [Young 1995]:

pedigree−id person−id father−id mother−id sex−of−person locus−value−1...locus−value−N

A pedigree ID may be up to 20 alphanumeric characters, and an individual's personal ID up to 12 characters. Missing values are denoted `x` (or `.`), and represented internally as a trait value of −9999.

Locus values for a binary trait are `y` (expresses trait), `n` (does not express trait). Sex takes the values `m` (male) and `f` (female), and may be missing. Alleles at a *marker* locus are integers between 1 and 999 or single letters. The alleles of a genotype may be separated by a slash. A pedigree file may contain a comment at any time, prefaced by `!` or #.

If only one parent of an individual is specified in the pedigree file, a dummy record and ID number for the other parent is generated by the program. Similarly, if both parental IDs are given, but there are no data

records for those IDs, dummy records will be generated. A pedigree founder is a person where both parental ID fields (columns 3 and 4) are set to missing, and a nonfounder a person where both parental IDs are given.

```
>> set loc q1 qua
>> set loc m1 mar
>> read pedigree inline
ped1 Bob Mark Alice m 10 a/b
;;;;
>> run
[Output omitted....]
>> write
 Writing  1  pedigrees:
!
!                     S
!                     e
!Ped Perso Fath Mothe x    q1       m1
!
ped1 Alice x    x     f     x       x/x
ped1 Mark  x    x     m     x       x/x
ped1 Bob   Mark Alice m   10.0000   a/b
```

As it happens, sex can also be coded as "1" (*m*) and "2" (*f*); binary traits as 1=n, 2=y.

A pedigree can either be read from a pedigree file (see below), or as in the example be inline in a script (or even entered from the keyboard). To read a script into Sib−pair, one can either send the contents of the script file directly to the program:

```
davidD@moonboom:~$ sib−pair < script.in
```

or start up Sib−pair, and use the "include" command:

```
davidD@moonboom:~$ sib−pair
|||| SIB−PAIR: A program for simple genetic analysis
|\/| Version : F95−DEV (06−Nov−2006)
|/\| Author  : David L Duffy (c) 1995−2006
|||| Job run : Tue Nov  7 15:30:54 2006

>> include script.in
```

If the "include" command is entered without specifying a file, a file chooser dialogue is opened. If "set gui" has been set, this is a graphical (AWT or GTK based) browser, but there is a backup text based browser:

```
>> include
[1] ./ [2] ../ [3] cavanaughex.in [4] ex.in [5] ghex.in [6] linclex.in
[7] liuex.in [8] longqtex.in [9] sib−pair.log [10] volgaex.in
[11] williamsex.in
choice> 7

Reading commands from "liuex.in".
```

These dialogues allow one to change directory etc.

Finally, one can type in the commands and data interactively. This is most likely if you are going to simulate data:

```
>> set loc m1 marker
>> sim ped 10 2 3 5
 Simulating  10  pedigrees of depth  2  generations with sibship size  3  to  5
>> run
```

Native format pedigree file

This follows the same format as seen in the inline example. This could be prepared using a editor or a spreadsheet, saving the file as a plain text file (in Windows, a ".txt" file).

There needs to be a Sib−pair script that identifies the type of each column of data in the file, and the inclusion of the "read pedigree <file_name>" command:

```
>> set loc q1 qua
>> set loc m1 mar
>> read pedigree example.ped
>> run
```

Merlin format pedigree file

The Merlin pedigree file format is actually completely compatible with that used by Sib−pair **except** that alleles at a genotype in a Merlin type file must be **numeric** (a nonnumeric value for an allele is regarded as a missing value). The dataset columns can either be described using the "set locus" command, or alternatively the Merlin ".dat" locus description file could be read using the "read locus merlin <file_name>" command:

```
>> read locus merlin merlin.dat

Read in names of  100 loci from locus file.

>> read pedigree merlin.ped
>> run
```

Linkage format pedigree file

Sib−pair can read both the "pre−makeped" and "post−makeped" Linkage format pedigree files. These are the formats used by a number of computer programs for genetic linkage and association analysis including the Linkage programs themselves (MLINK, ILINK, LINKMAP etc), Genehunter, Allegro, SUPERLINK, and UNPHASED. The full Linkage format is described online in several references.

Again, you can provide a description of the dataset columns using the "`set locus`" command, or read in the Linkage format ".dat" locus description file.

```
>> read locus linkage m_ger06.loc

Read in names of   53 loci from locus file.

>> read linkage m_ger06.pre
>> run
```

FREQUENTLY USED COMMANDS

Although there are a number of Sib−pair commands that can be used without having a dataset in memory, most are targetted at data analysis.

`set locus`

As already mentioned, this command declares a variable. Each "`set locus`" command declares the name and type of another column of data reading from left to right in the dataset.

```
>> set loc q1 qua
>> set loc m1 mar
```

The above two statements make the first data column (column 6 of the pedigree file) a quantitative trait called "q1", and second and third data columns (columns 7 and 8 of the pedigree file) the first and second alleles of an autosomal codominant locus called "m1".

The "`set locus`" declaration can be extended to give a genetic map position for the locus (in centiMorgans), and a 40 character comment describing the locus. Several commands can search the comments for particular strings (including wild card matches). For a trait as opposed to a marker, the position is usually unknown, but a place holder is needed if one wishes to write a comment.

```
>> set loc q1 qua  .  First quantitative locus
>> set loc m1 mar 4.6 First marker locus
>> list

Locus           Type Position
--------------- ---- ----------
q1               q      6         First quantitative locus
m1               m      7--   8   First marker locus

Number of marker loci=    1
Bonferroni corr. 5%  =    0.050000
Bonferroni corr. 1%  =    0.010000
Bonferroni corr. 0.1%=    0.001000

>> ls
q1* m1
 1  active traits;  1  active markers.
```

list

The "list" command lists the currently declared loci in the dataset, while the "ls" command gives an abbreviated listing.

The listing can be limited to a subset of loci:

```
>> lis rs*00*

Locus           Type Position
--------------- ---- ----------
rs1800420        m    286-- 287   rs1800420 25763792 G/G
rs1800419        m    288-- 289   rs1800419 25770133 A/A
rs1004611        m    290-- 291   rs1004611 25770873 -
rs1800418        m    298-- 299   rs1800418 25789931 S/S
rs1800417        m    300-- 301   rs1800417 25789974 I/T
rs1800416        m    308-- 309   rs1800416 25844889 A/A

Number of marker loci=    6
Bonferroni corr. 5%  =    0.008512
Bonferroni corr. 1%  =    0.001674
Bonferroni corr. 0.1%=    0.000167

>> lis $a

Locus           Type Position
--------------- ---- ----------
cmm              a     26
invasive         a     36
```

Within the "`list`" command, loci selection can be via marker name, where matching can be by wildcards, or by locus type:

`$a` All active dichotomous traits

`$q` All active quantitative trait

`$h` All active haploid markers

`$m` All active autosomal markers

`$x` All active X−chromosome markers

The latter can be further modified by addition of an ordering flag

`m` Genetic map order rather than column order

`r` Reverse of column (or map `mr`) order

run

The "run" command reads the pedigree data from the file (or script) into the dataset in memory. As it carries this out, it:

Checks for duplicate records

Checks for impossible pedigree relationships (eg own father)

Checks sex of parents

Creates extra pedigree records as required

Sorts the pedigree by generation number

Checks for unconnected components within the pedigree

Tests and reports Mendelian errors

Tests that the reported sexes are consistent with sex−linked marker genotypes

Tests that monozygotic twins are concordant at all markers

Generates legal values for all missing genotypes within the pedigree − used to start MCMC algorithms.

Impute missing genotypes if requested ("set imputation on")

```
Pedigree     Individual  Sex  Post.Pr(M)   X−marker hets
-----------  ----------  ---  ----------   -------------
04620        0462030      f    1.000000       0/  19
09901        0990151      f    1.000000       0/  19
10157        1015704      m    0.000000      14/  19
23204        2320403      m    0.000000      16/  18
25122        2512203      m    0.000000      13/  19
25122        2512204      f    1.000000       0/  19
29344        2934401      f    1.000000       1/  19
29344        2934450      m    0.000000      12/  19
32802        3280203      m    0.000000      14/  19
32802        3280204      f    1.000000       0/  19

Designated     Sex inferred via sex−linked markers
Sex          Likely Male    Uncertain   Likely Female
----------   -----------    ---------   -------------
 Male             721          1806             5
 Unknown            0           145             0
 Female             5          2136           811
```

The output from the testing of sex includes an estimate of the posterior probability an individual is male, if the probability of an inconsistency exceeds 99.9%. A count of the heterozygote genotypes for each person is also provided. If a sex informative marker such as amelogenin is available, this can be incorporated in the analysis.

```
NOTE:  inconsistency due child 34114−3411402 at locus GATA31F01P {145/153}

X−linked locus "GATA31F01P"
−−−−−−−−−−−−−−
Sibship: 34114−3411403 x 34114−3411404

Inconsistency between sibling genotypes.

                      [3411403]           (3411404
                        x/−               145/153
                         |                   |
                         +=========+=========+
                                   |
                +----------+---------+---------+---------+
                |          |         |         |         |
            (3411401) (3411402) (3411451) [3411452] [3411453]
            145/149   145/153   145/153   153/−     153/−
```

Each Mendelian error detectable at a nuclear family level gives rise to a description of the individual where the error was detected, as well as a pedigree drawing showing all the relevant genotypes.

```
NOTE:  Mendelian inconsistency in pedigree 02590 at X−linked locus "GATA31E08".

X−linked locus "GATA31E08"
──────────────
Sibship: 02590−0259001 x 02590−0000005

Multigenerational inconsistency between genotypes.

                 [0259003] (0259004)
                   x/−     242/242
                    |         |
                    +====+====+
                         |
                      [0259001]           (0000005
                        x/−                 x/x
                         |                   |
                         +=========+=========+
                                   |
                              +────+────+
                              |         |
                           [0259008] (0259009)
                           230/−     230/230

ID         Count    Problem phenosets
────────── ──────── ─────────────────

Paternal Gparents
0259003           3 230/− 242/− +/−
0259004       Typed 242/242

Paternal Uncles/Aunts
0259002           1 242/−

Father
0259001     Problem 242/−

Mother
0000005     Problem 230/230 230/242 230/+ 242/242 242/+ +/+

Children
0259008       Typed 230/−
0259009       Typed 230/230

Parent 02590−0259001 cannot carry the "230" allele found in child 02590−0259008.
Parent 02590−0259001 cannot carry the "230" allele found in child 02590−0259008.
Parent 02590−0259001 cannot carry the "230" allele found in child 02590−0259009.
Parent 02590−0259001 cannot carry the "230" allele found in child 02590−0259009.
```

A multigenerational Mendelian inconsistency leads to printing of a table of pedigree members' observed or possible genotypes, along with (where possible) a statement of which transmission lead to the calling of the error. The pedigree drawing does not include uncles and aunts, so their genotypes must be found in the table. The above example is for a sex−linked marker.

`set errordrop on`

If any errors are encountered during checking, the **default action of the program is to stop**. If the "`set errordrop`" command has been issued (on), then the pedigree, or the genotypes at the appropriate marker for the pedigree will be set to missing.

out

Usually, Sib−pair output goes to the screen in interactive mode. To save output, I usually run Sib−pair in batch mode from the command line, reading in a control script, and piping the output to a file:

```
> sib−pair < script.in > script.out
```

During an interactive session, output can be diverted to a file by the "output" command. "output <filename>" saves subsequent output to the file filename, and "output" turns off the diversion, and resumes printing to the screen.

```
>> output script.out
>> describe snps
>> output
```

describe

This command produces summary statistics for the different variables. It can produce various amounts of output.

```
>> des snp

Marker          NAll  Allele(s)    Freq   Het    Ntyped
--------------- ---- -----------   ------ ------ ------
rs977588           2  G    (T)     0.4134 0.4851  3674
rs977589           2  A    (G)     0.4548 0.4960  3818
rs2311843          2  A    (G)     0.1474 0.2514  3815
rs1800415          1  G            1.0000  −      3828
rs7164127          1  C            1.0000  −      3828
rs1800414          2  G    (A)     0.0034 0.0068  3828
```

The "`describe snps`" command produces a one−line summary for each marker giving the marker name, number of alleles, the allele names, the minor allele frequency if there are two alleles, the expected heterozygosity and number of individuals genotyped at that marker.

```
>> des rs977588

------------------------------------------------
Allele frequencies for locus "rs977588"
------------------------------------------------
   Allele  Frequency   Count  Histogram
     G       0.4134     3038  ********
     T       0.5866     4310  ************

Number of alleles    =    2
Heterozygosity (Hu)  =    0.4851
Poly. Inf. Content   =    0.3674
4 Neff mu (SSMM)     =    1.68136183
Number persons typed =   3674 ( 72.4%)
```

One obtains more information by not using the "`snp`" modifier.

For a quantitative trait, one would obtain,

```
>> des logIgE

------------------------------------------------
Summary statistics for trait "logIgE"
------------------------------------------------

Descriptive Stats       All     Founders  Nonfounders
-----------------------------------------------------
Means                4.4033       3.8716       4.6209
Variances            2.3192       1.8137       2.3647
Stand Devs           1.5229       1.3467       1.5378
Maxima               8.4338       8.3547       8.4338
Minima               2.0149       2.0149       2.0149
No. obs           1550          450         1100
No. missing       4076         2044         2032
```

First summary statistics for the entire sample, the pedigree founders, and pedigree nonfounders.

```
-------------- Familial correlations (pairwise) --------------
Rel 1   Rel 2    Std Dev 1    Std Dev 2   Correlation  N Pairs
--------------------------------------------------------------
Husband Wife         1.2812       1.3113       0.0063      197
Gparent Gchild       0.0000       0.0000       0.0000        0
Halfsib Hsib         1.6126                   −0.0417       19
Parent  Off          1.3257       1.6067       0.2342     1208
Fullsib Fsib         1.5332                    0.2986      967

Father  Son          1.2363       1.5452       0.1765      273
Father  Dau          1.2571       1.6201       0.1601      259
Mother  Son          1.3900       1.5636       0.3145      356
Mother  Dau          1.3673       1.6784       0.2448      320

Brothers             1.5042                    0.3265      263
Sisters              1.5670                    0.3937      313
Brother−Sister       1.4998       1.4969       0.1850      391
```

Then family correlations for various types of relative pair. pedigree nonfounders.

```
WLS estimates of heritability (approx SE)

Heritability  =     0.4662 (0.0008)
Dominance (d2)=     0.2618 (0.0023)

Fain sibship variance test
--------------------------
No. sibships  =   387
Intercept     =     0.1031 (ase=    0.3792)
Slope         =    −0.1242 (ase=    0.0793)
t value       =     1.5658 (df=385, P=0.0591)
```

And finally an approximate estimate of trait heritability, based on the pairwise correlations, and the Fain sibship variance test, which tests the regression of the midparent value on the offspring variance. If the latter is significant, this heteroscedasticity may reflect effects of a major gene, gene by environmental interaction, or an inappropriate data transformation.

The results for a dichotomous trait are less extensive:

```
>> des hiIgE

------------------------------------------------
Segregation ratios for trait "hiIgE     "
------------------------------------------------

Total sample   All       Fndrs     Nonfndrs
-----------------------------------------
   Aff/Tot   686/1550  128/ 450  558/1100
   Prop Aff     0.443     0.284     0.507
   Missing       4076      2044      2032

Mating Type     UxU       UxA       AxA
-----------------------------------------
   Matings        102        79        16
   Aff/Tot   118/ 263  126/ 201   30/  38
   Prop Aff     0.449     0.627     0.789

Relative pair  RecRisk   Aff−Aff   Aff−UnA
-----------------------------------------
   Marital      0.288        16        79
   Gparent      0.000         0         0
   Halfsib      0.769        10         6
   Par−Off      0.459       230       542
   Fullsib      0.625       312       375
```

The tables give the trait prevalence in founders, nonfounders and overall, the segregation ratios (unadjusted for ascertainment) −− the "davies" command gives adjusted estimates and standard errors, and the recurrence risks for different classes of relative pair.

set plevel

The "set output" or "set plevel" command controls the verbosity of the printed output of the program. The usual level is "0", but it can be set both lower and higher than this. A `plevel` of "1" used to be standard, but this produces large amounts of output to be read. At that level, however, the details of the actual statistical tests performed are a lot clearer. Setting the `plevel` to "2" will give output from each iteration of an algorithm, such as likelihood maximization or simulation.

Setting the `plevel` to "−1" discards a number of warnings: Mendelian errors are reported as a single line of output per error, and minor pedigree problems (unrelated members etc) are not flagged.

hwe

The "hwe" command carries out a test for Hardy−Weinberg Equilibrium (HWE) on all (or a specified subset of markers). It calculates the likelihood ratio test statistic (LRTS) for HWE, but simulates P−values to allow for relatedness of pedigree members. All pedigree members therefore contribute to the test. This differs from the approach of several programs that test for HWE only in pedigree founders or unrelated individuals. The test is therefore a combined test of Hardy−Weinberg disequilibrium in founders and segregation distortion in nonfounders. The "founders" keyword restricts the analysis to founders only. And for diallelic markers, both the LRTS and HWE exact test P−values are calculated (the latter is only printed if plevel > 0).

```
>> hwe

------------------------------------------------
Hardy-Weinberg equilibrium for marker loci
------------------------------------------------

Marker     Typed  Genos  Chi-square Asy P  Emp P  Iters
---------- ------ ------ ---------- ------ ------ ------
GATA52B03    1574     36       51.3 0.0368 0.0597    201 HWE +
AGAT144      1562     36       28.0 0.7919 0.9524     21 HWE .
GATA175D03   1499     78       53.6 0.9805 0.2273     88 HWE .
ATA28C05     1538     28       26.4 0.4979 0.4651     43 HWE .
GATA124E07   1598    120      112.7 0.6441 0.1439    139 HWE .
GATA027      1569     21       12.9 0.8812 0.7143     28 HWE .
GATA69C12    1585     36       27.1 0.8260 0.2941     68 HWE .
GATA144D04   1519     55       51.6 0.5667 0.2985     67 HWE .
GATA72E05    1534     36       29.1 0.7493 0.6452     31 HWE .
GATA31D10    1577     36       34.5 0.4902 0.5882     34 HWE .
GATA31F01    1580     66       62.6 0.5616 0.2500     80 HWE .
GATA172D05   1589     36       37.2 0.3701 0.3846     52 HWE .
GATA48H04    1538     36       18.3 0.9912 0.7407     27 HWE .
GATA165B12   1590     15       26.5 0.0223 0.0249    201 HWE +
ATCT003      1529     45       63.9 0.0267 0.0597    201 HWE +
GATA31E08    1514     36       44.0 0.1421 0.3571     56 HWE .
DXS998       1602     21       12.9 0.8802 0.7143     28 HWE .
TATC043      1584     45       34.6 0.8431 0.4878     41 HWE .
TTTA062      1587     21       40.5 0.0043 0.0448    201 HWE *
```

```
>> set ple 1
>> hwe TTTA062

−> hwe TTTA062


−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
Hardy−Weinberg equilibrium for marker loci
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−

  −−−−−−−− Observed Genotypes at "TTTA062   " −−−−−−−−
       Genotype          Observed    Expected  Deviate
  −−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
        136/136          0 (0.000)        1.5     −1.6
        136/140          0 (0.000)        0.7     −1.0
        140/140          0 (0.000)        0.1     −0.2
        136/144         13 (0.015)        9.7      1.0
        140/144          3 (0.004)        2.4      0.5
        144/144         18 (0.021)       16.2      0.5
        136/148          6 (0.007)       12.1     −1.9
        140/148          5 (0.006)        3.0      1.1
        144/148         31 (0.036)       40.2     −1.5
        148/148         33 (0.039)       24.9      1.5
        136/152         55 (0.064)       41.3      2.0
        140/152          7 (0.008)       10.2     −1.0
        144/152        148 (0.173)      137.3      0.9
        148/152        171 (0.200)      170.4      0.1
        152/152        270 (0.316)      291.1     −1.2
        136/156          1 (0.001)        4.0     −1.7
        140/156          0 (0.000)        1.0     −1.2
        144/156          5 (0.006)       13.3     −2.7
        148/156         19 (0.022)       16.5      0.6
        152/156         65 (0.076)       56.4      1.1
        156/156          5 (0.006)        2.7      1.2
     Male Haplotype      Observed    Expected  Deviate
        136/−           26 (0.036)       30.3     −0.8
        140/−           10 (0.014)        7.5      0.9
        144/−          100 (0.137)      100.7     −0.0
        148/−          119 (0.163)      125.0     −0.5
        152/−          439 (0.600)      427.1      0.6
        156/−           38 (0.052)       41.4     −0.5
  −−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
          Total       1587 (1.000)

       Number of genotypes =1587 ( 732 male)
  Hardy−Weinberg LR chi−sq =  40.5
Nominal degrees of freedom =  20
           Nominal P−value =   0.0043
      Equalled or exceeded by =   7/  201 simulated values (0.0348)
 Mean (Var) simulated chi−sqs =  24.1 (  66.7)
```

In this example, the markers are X−linked, so the test includes a contribution due to allele frequencies differences between males and females. There are two sets of P−value, the asymptotic P−values ignoring relationships and the empirical P−values obtained from gene−dropping using the observed pedigree structure and allele frequencies. The "Iters" column (and the equivalent line in the verbose output) gives the number of simulations (replicates of the dataset) used. The maximum number of simulations defaults to 200, but if the P−value is nonsignificant, the sequential approach used will stop early.

The other test for HWE that Sib−pair provides compares the observed to expected heterozygosities:

```
>> homoz
```

```
------------------------------------------------
Marker homozygosity in all typed individuals
------------------------------------------------

Marker        N    Obs    Exp    Fis     Z  Emp P  Iters
---------- ---- ------ ------ ------ ------ ------ ------
TTTA062     855 0.3813 0.3936 −.0203   −0.7 0.6452     31 HOM .
```

This command, "homoz" can also be applied to homozygosity mapping or Hardy−Weiberg disequilibrium mapping by specifying a dichotomous trait: "homoz <trait>".

Finally, use of the "table" command to crosstabulate a diallelic marker by another locus gives an exact HWE test for each stratum.

```
>> tab q1 rs8004738

------------------------------------------------
Cross−tabulation of "q1" ... "rs8004738"
------------------------------------------------

                     rs8004738
q1               C/C          C/T          T/T     Allele Freq  Exact HWE−P
           ------------------------------------------------------------------
   1.00000    271 (.230)   620 (.527)   285 (.242)  0.4940  0.5060    0.0705
   2.00000    151 (.227)   342 (.515)   171 (.258)  0.4849  0.5151    0.4383
```

nuclear

There are several Sib−pair commands that can be used to transform the pedigree structure of a dataset. The simplest such command ("nuclear") breaks down a larger pedigree into its constituent nuclear families:

```
>> nuc

Dividing  pedigrees into nuclear families.
Individuals are duplicated as necessary.
 Reread    16  pedigrees,  90  individuals (0.00 s).
 Dataset occupies 0.010 Mb.
Extracted     16 nuclear families.
```

The "grandparents" modifier adds in the grandparents for each nuclear family, such that they will be "CEPH" type families.

Another command for pedigree manipulation is "join" which connects pedigrees together via common individual IDs. This is useful if you wish to reconnect subsets of nuclear families created using the "nuclear" command. Thus you can subdivide a pedigree into the largest blocks analyzable by another program; such as MERLIN.

The other commands for pedigree manipulation include "prune", which removes pedigree members who are uninformative for a particular trait locus (unknown status themselves, and not connecting informative sets of relatives); and "case" which extracts the unrelated cases and controls from a pedigree, discarding other pedigree members.

select

While the "keep|drop" and "undrop" commands allow you to choose the subset of data columns −− ie variables, the "select" and "unselect" commands allow the selection of rows of data −− pedigrees. Selection is either by the desired pedigree names, or using a "where" clause; which can be any logical expression.

```
>>  select containing 2 where anytyp

Selecting pedigrees to contain   2 or more individuals where "anytyp":
Number of pedigrees selected=   15 (  334 individuals)
```

The previous command selected only those pedigrees where two or more members are genotyped at any active marker (we could have used the "keep|drop" command to restrict the test to a subset of all the markers). If genotyping is patchy, we might have instead used:

```
>>  unselect
>>  select containing 2 where commar > 2
```

where commar is a function counting the maximum number of markers an individual shares with any of their relatives.

Association analysis

This can be between markers (linkage disequilibrium, LD) or between a trait locus and a marker.

Intermarker association

The "`disequilibrium`" command defaults to calculating measures of linkage disequilibrium for pairs of codominant marker loci. It tests both intragametic and intergametic association.

```
>> dis 2 2
  9 genotype counts>
91 147 85
32 78 75
5 17 7
Modelling   9 unphased genotypes (N=  537).

  Haplotype  Prop      95% CL           D        D'
  ------------------------------------------------------
   1   1   0.3822   0.3503−−0.4169    0.0234    0.2231
   1   2   0.3916   0.3594−−0.4266   −0.0234   −0.2231
   2   1   0.0815   0.0664−−0.1001   −0.0234   −0.2231
   2   2   0.1448   0.1244−−0.1684    0.0234    0.2231

  Number of genotypes used =       537
    LD Model LR Chi−square =       12.81 (df=   5, P=0.0252)
       LR Chi−square (D=0) =        8.01 (df=   1, P=0.0046)
  Hedrick weighted mean D' =        0.2231
                 r−squared =        0.0126
```

This analysis read a 3x3 table of genotype counts from the command line −− the "`dis` 2 2" lead Sib−pair to expect data for two diallelic markers. Sib−pair fitted loglinear models to the counts, the critical one allowing linkage disequilbrium between the two loci (D' > 0). It tested this model against that assuming complete linkage equilibrium, giving a significant LRTS=8.01. Since these are both diallelic markers, Sib−pair also printed the r^2 measure of LD as well as D'.

For family data, Sib−pair uses parents where available to infer haplotype phase, and combines information from phased and unphased genotypes. Pedigree members are discarded as appropriate, so that the resulting tables of genotype counts come from unrelated individuals.

```
>>  set ple 2
>>  dis A308G rs3897937

->  dis A308G rs3897937


-------------------------------------------------
Inter-marker allelic association analysis
-------------------------------------------------


Assoc for locus "A308G     " c. locus "rs3897937 "
-------------------------------------------------

Modelling  10 phased genotypes (N=  319) and   9 unphased genotypes (N= 1619).
And   4 male haplotypes (N= 1360).

Unphased Genotypes  Observed  Expected  Deviance
  1/1     1/1           774.    769.2      0.2
  1/1     1/2           495.    503.0     -0.3
  1/1     2/2           101.     82.2      2.0
  1/2     1/1             0.      0.0     -0.0
  1/2     1/2           175.    190.5     -1.1
  1/2     2/2            55.     62.3     -0.9
  2/2     1/1             0.      0.0      0.0
  2/2     1/2             0.      0.0     -0.0
  2/2     2/2            19.     11.8      1.9
Phased Genotypes    Observed  Expected  Deviance
  1  1;  1  1           149.    151.6     -0.2
  1  1;  2  1           100.     99.1      0.1
  1  1;  2  2            21.     16.2      1.2
  2  1;  1  1             0.      0.0     -0.0
  2  1;  1  2             0.      0.0     -0.0
  2  2;  1  1             0.      0.0      0.0
  2  1;  2  1            41.     37.5      0.6
  2  1;  2  2             8.     12.3     -1.2
  2  2;  2  1             0.      0.0     -0.0
  2  2;  2  2             0.      2.3     -2.2
Male Haplotypes     Observed  Expected  Deviance
     1 1               952.    937.4      0.5
     1 2               278.    306.5     -1.7
     2 1                 0.      0.0     -0.0
     2 2               130.    116.1      1.3

  Haplotype  Prop      95% CL           D        D'
  ---------------------------------------------------
 A   A     0.6893   0.6686--0.7105    0.0588    1.0000
 A   G     0.2254   0.2131--0.2383   -0.0588   -1.0000
 G   A     0.0000   0.0000--  +Inf   -0.0588   -1.0000
 G   G     0.0854   0.0779--0.0936    0.0588    1.0000

  Number of genotypes used =      3298
    LD Model LR Chi-square =       22.61 (df=  17, P=0.1622)
       LR Chi-square (D=0) =      898.94 (df=   1, P=0.0000)
  Hedrick weighted mean D' =        1.0000
                 r-squared =        0.2070
```

In this example, the SNPs are on the X−chromosome, so males provide haplotype frequencies directly, but no information about intergametic association. There is "complete" LD, but not "perfect" LD. The overall model goodness−of−fit test is not significant, thus suggesting an absence of intergametic effects.

If one specifies the names of more than two autosomal markers, or two or more autosomal markers and a

binary trait, then the command estimates haplotype frequencies using a log−linear model.

```
>> dis G2215A ACE_ID G2350A hiace

−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
Inter−marker allelic association analysis
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−

Trait:   hiace(2)
Markers: G2215A(2) ACE_ID(2) G2350A(2)

Haplotype  n          y
−−−−−−−−   −−−−−−−−−  −−−−−−−−−
1 1 1      0.0000     0.0000
2 1 1      0.6711     0.2975
1 2 1      0.0000     0.0000
2 2 1      0.0000     0.0000
1 1 2      0.0000     0.0000
2 1 2      0.0000     0.0000
1 2 2      0.3263     0.6994
2 2 2      0.0026     0.0032

hiace         190         158

            Number of loci =         3
 No. genotyped individuals =       348
 No. obs. unique genotypes =         8
  Stratified LD Chi−square =     18.26 (df=  48, P=1.0000)
    Association Chi−square =     98.91 (df=   2, P=0.0000)

NOTE:  Degrees of freedom calculation for association test assumes only
          3 haplotypes to be present in the population.
```

The algorithm used is not optimized for either large numbers of alleles or large numbers of loci.

Trait−marker assocation

There are five specialized trait locus association commands:

`assoc`	Total association; FBAT (Family−based Association Test)
`tdt`	Transmission−disequilibrium tests
`hrr`	Haplotype relative risk test
`schaid`	Schaid's TDT
`sdt`	Sibship disequilibrium test

These are supplemented by the "`regression`" command, which also allows gene−dropping for pedigrees (via the `simulate` modifier), and commands for "*measured genotype*" models −− "`varcom`" and "`fpm`". And as noted above, the "`homoz`" command can also be a powerful test of association in a *case−only* analysis.

General association

The "`assoc`" command and `regression tests` can be used for samples of unrelated individuals and family data. They deal with familial correlated data by calculating gene−dropping simulated P−values. The type of gene−dropping currently used is not conditional on marker IBD, so although the resulting test has the correct *size* in a statistical sense, it can detect linkage as well as pure association in larger pedigrees:

```
>> ass AD

------------------------------------------------
Allelic association testing for trait "AD"
------------------------------------------------

Marker     Typed  Allels Chi−square Asy P  Emp P  Iters
---------- ------ ------ ---------- ------ ------ ------
D14S52         21      7        4.7 0.5890 0.2793    179 AssX2−HWE .
D14S52          6      7        0.5 1.0000 0.8772     57 RC−TDT    .
D14S43         21      7       12.6 0.0498 0.0064   5001 AssX2−HWE *
D14S43          2      7        2.9 0.5171 0.2404    208 RC−TDT    .
D14S53         20      6       11.0 0.0516 0.0054   5001 AssX2−HWE *
D14S53          3      6        1.0 1.0000 0.4237    118 RC−TDT    .
```

These results are from three Volga German Alzheimer's pedigrees described in Figure 1 of Schellenberg et al [1992]. These families harbour mutations in the presenilin 1 gene. At 72.673 Mbp from the pter of chromosome 14, this is 1.34 Mbp from D14S43, (the closest of the three microsatellite markers used in that paper), which gave a linkage lod score of 7.

```
>> keep AD D14S43
>> select pedigree L
>> set iterations 90000
>> set plevel 1
>> assoc AD
------------------------------------------------
Allelic association testing for trait "AD"
------------------------------------------------

  ---- Association Analysis for "D14S43    "-----
    Allele   Affected    Unaffected    Total    Dev
  ------------------------------------------------
   159       11 (.786)      0 (.000)      11    2.3
   179        0 (.000)      1 (.167)       1   −1.5
   181        2 (.143)      1 (.167)       3   −0.2
   183        1 (.071)      0 (.000)       1    0.7
   185        0 (.000)      2 (.333)       2   −3.1
   187        0 (.000)      2 (.333)       2   −3.1
  ------------------------------------------------
   Total     14             6             20

       No. trait(+) marker(−) =    20
       No. trait(+) marker(+) =    10
                 Fis, Fit, Fst =   −.1765   0.2683   0.3780
   Contingency Pearson chi−sq =  16.8
   Nominal degrees of freedom =   5
              Nominal P−value =   0.0048
      Equalled or exceeded by =   4/90001 simulated values (0.0000)
 Mean (Var) simulated chi−sqs =   2.8 (   4.5)
```

We can obtain a more impressive result by restricting our analysis to one pedigree (pedigree L), where the 159 allele cosegregates with the disease. The point is that we are observing a pure test of cosegregation/linkage, that is correctly modelling the transmission of the marker allele through the pedigree.

By contrast, in the study of Liu et al [1996], 15 late−onset Alzheimer's pedigrees were genotyped at the APOE locus. Evidence of linkage to APOE was weak, but there was strong evidence of allelic association.

```
>> ass AD
------------------------------------------------
Allelic association testing for trait "ad"
------------------------------------------------

Marker     Typed  Allels Chi−square Asy P  Emp P  Iters
---------- ------ ------ ---------- ------ ------ ------
apoe          163      3       18.4 0.0001 0.0001  50001 AssX2−HWE ***
```

Since there are few alleles (and the result was strongly significant), it is worth looking at the genotypic effects.

```
>> set plevel 1
>> ass AD gen

−> ass ad gen

------------------------------------------------
Allelic association testing for trait "ad"
------------------------------------------------
NOTE:  Genotypic rather than allelic association test.

  ---− Association Analysis for "apoe      "−----
  Genotype   Affected    Unaffected    Total    Dev
  ------------------------------------------------
   2/2        0 (.000)      0 (.000)       0    0.0
   2/3        1 (.023)      7 (.058)       8   −1.0
   3/3        7 (.163)     58 (.483)      65   −2.9
   2/4        1 (.023)      5 (.042)       6   −0.6
   3/4       23 (.535)     38 (.317)      61    2.0
   4/4       11 (.256)     12 (.100)      23    2.4
  ------------------------------------------------
   Total     43           120            163

       No. trait(+) marker(−) =   168
       No. trait(+) marker(+) =    81
   Contingency Pearson chi−sq =  18.7
   Nominal degrees of freedom =   4
              Nominal P−value =   0.0009
      Equalled or exceeded by =  20/28061 simulated values (0.0007)
 Mean (Var) simulated chi−sqs =   4.1 (   7.6)
```

The genotypic test simulation P−value is also highly significant. The greatest risk of disease was associated with the APOE*E3/APOE*E4 and APOE*E4/APOE*E4 genotypes

```
>> regress onset = apoe weibull AD sim

−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
Weibull  regression analysis of trait "onset"
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
Censoring variable: ad.

    Variable         Beta    Stand Error        t−Value
  −−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
  Intercept       43.6621         1.4266        30.6064 ***
  apoe*3          −0.7437         0.7265         1.0237 .
  apoe*4          −1.8245         0.7385         2.4705 *

No. usable observations =    162      ( 48.5%)
No. of uncensored times =     43      ( 26.5%)

Weibull shape parameter =      9.4276
Number of iterations    =     91
Model LR Chi−square     =    111.5137 (df= 159)
Akaike Inf. Criterion   =    117.5137

ERROR: IRLS failed (perhaps due to separation).

Due to IRLS failure, discarded Pseudosample      128

Gene−dropping association test for "apoe"
Equalled or exceeded by =    1/ 50001 simulated values (0.0000)
Mean (Var) sim deviance =    123.6625 (      1.9032)
```

We can also carry out a Weibull−regression based survival analysis (see above Table) on the ages−at−onset in this set of pedigrees and confirm the association. Out of over 50000 simulations, about 50 give an error message (probably due to one or more genotypes not appearing in cases or controls) −− these were discarded and replacement samples drawn.

An alternative approach is to generate residuals from a survival analysis and carry out association analysis on these:

```
>> set loc adres qua
Creating new variable "adres".

>> adres=onset
Operating on pedigree file

Recoded    165 values.

>> kap adres ad res
```

```
------------------------------------------------
Kaplan−Meier survivor function for "adres"
------------------------------------------------
"ad" is outcome (censoring) trait.

Replacing value of "adres" with nonparametric residual.

 Age−at−onset   Failed  Riskset   H(t)   S(t)    ase
 ------------------------------------------------------
      52.0000        1      111 0.0090 0.9910 0.0090
      57.0000        1       98 0.0192 0.9809 0.0134
      58.0000        1       95 0.0297 0.9706 0.0168
      59.0000        1       94 0.0404 0.9602 0.0195
      60.0000        4       91 0.0843 0.9180 0.0278
      61.0000        2       84 0.1081 0.8962 0.0312
      62.0000        1       79 0.1208 0.8848 0.0328
      65.0000        5       74 0.1884 0.8250 0.0400
      67.0000        4       66 0.2490 0.7750 0.0447
      68.0000        1       58 0.2662 0.7617 0.0459
      69.0000        3       56 0.3198 0.7209 0.0491
      70.0000        4       50 0.3998 0.6632 0.0530
      72.0000        5       37 0.5349 0.5736 0.0591
      73.0000        1       30 0.5683 0.5545 0.0601
      76.0000        5       24 0.7766 0.4389 0.0662
      79.0000        2       15 0.9099 0.3804 0.0691
      80.0000        1       12 0.9933 0.3487 0.0702
      83.0000        1        7 1.1361 0.2989 0.0758

 H(t) = Nelson−Aalen estimator of integrated hazard
 S(t) = Kaplan−Meier estimator of survivor function

   43 affecteds and   122 unaffecteds used

>> ass adres gen

>> ass adres gen


------------------------------------------------
Allelic association testing for trait "adres"
------------------------------------------------
NOTE:  Genotypic rather than allelic association test.

  ------ QTL Association with "apoe      " -----
  Genotype   Gtypic Mean     Stand Error   Count
  ---------------------------------------------
   2/2             0.0000         0.0000       0
   2/3            −0.3562         0.2815       8
   3/3            −0.3421         0.0988      65
   2/4            −0.6132         0.3250       6
   3/4             0.1996         0.1003      63
   4/4             0.3937         0.1660      23
  ---------------------------------------------
  Total           −0.0433         0.8485     165

 No. trait(+) marker(−)  =      0
 No. trait(+) marker(+)  =    165
 Model Mean Square       =      3.3907 (df=   5)
 Mean Square Error       =      0.6339 (df= 160)
 Likelihood ratio test   =     25.0743
 Nominal P−value         =      0.0000
 Equalled or exceeded by =   3/50001 simulated values (0.0001)
 Mean (SD) simulated MSE =      0.7222 (      0.0114)
```

In the table above, the use of Sib−pair's "kaplan−meier" command provides the Kaplan−Meier estimate of the survival curve, as well as replacing the age−at−onset trait by the transformed martingale residuals if the "residuals" keyword is included. A standard ANOVA is performed, and gene−dropping used to give a correct P−value given the relatedness of the sample. Even after transformation (following Therneau), the residuals are not "nicely" distributed, so the gene−dropping is doubly necessary.

```
>> his adres

------------------------------------------------
Mixture distributions for trait "adres"
------------------------------------------------

 Intvl Midpt  Count   Histogram
 ---------------------------------------------------
     −1.4066     11   ***********
     −1.2463      4   ****
     −1.0033      7   *******
     −0.8016     17   *****************
     −0.6138      3 | ***
     −0.3984     10 | **********
     −0.1967     17 | *****************
      0.0049     57 + ************************************************
      0.2426      5   *****
      0.4082      0
      0.6099      6   ******
      0.7957      4   ****
      1.0131      4   ****
      1.2148      9   *********
      1.4164      0
      1.6181      3   ***
      1.7648      4   ****
      2.1213      1   *
      2.2561      1   *
      2.4378      1   *
      2.7271      1   *

Filliben correlation =        0.8037 (P=0.000)

Poissonness test Z   =     −582.9685 (P=1.000)
Median (IQR)         =        0.0000 (      −0.6138 −−        0.0068)
Symmetry test J(.02) =        0.4142 (P=0.000)
```

Association analysis using the " regress" or "fpm" commands can also include inferred marker genotypes for untyped members of the pedigree via two different methods. One can estimate the expected gene dose for the marker using the "gpe" command, and use this instead of the observed genotypes in the regression. This does not allow for residual family correlation (unless "fpm" is used).

Alternatively, by issuing the "set analysis imputed" command, each subsequent "regress" call will cause reimputation of missing genotypes (at the marker included in the model), with these imputed genotypes being included in the regression analysis. It is then necessary to fit the model repeatedly and average over test statistics (*multiple imputation*) so as to correctly represent the uncertainty in the estimates for the unobserved genotypes. This is carried out automatically by the replicates option of the "regress" command.

Family based tests of association

Tests such as the transmission−disequilibrium test (TDT), and the Family−based Association test (FBAT) are tests of joint linkage and association. In practice, they are usually seen as association tests that are insensitive

to confounding due to ethnic stratification in a sample of families. Sib−pair automatically performs a simulation−based version of the FBAT whenever the "assoc" command is run. The gene−dropping is performed *conditional on parental genotypes* (CPG) and conditional on offspring genotypes needed to impute missing parental genotypes.

```
>> inc liuex.in
>> set plevel 1
>> ass AD

−> ass ad
```

Returning to the dataset from Liu et al [1996], we obtain:

```
−−−−−−−− Combined transmission test for "      apoe" −−−−−−−−
  Allele   Affected   Unaffected   Total   E(Aff)    Z    P
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
    2        2 (.03)      6 (.10)      8    3.7   −1.3 0.1830
    3       28 (.41)     37 (.60)     65   34.5   −2.2 0.0306
    4       38 (.56)     19 (.31)     57   29.8    2.7 0.0073
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
  Total     68           62          130

                  marker(−) =     3
      No. trait(+) marker(+) =    65
         No. useful sibships =    13
Global association statistic =   2.2
          Degrees of freedom =   2
     Equalled or exceeded by =  20/ 1349 simulated values (0.0148)
Mean (Var) simulated chi−sqs =   0.5 (   0.3)
```

The second column records the number of times that the different alleles were transmitted from a parent to an affected child. The third column records the number of times the same alleles were transmitted to an unaffected child. Since the latter does not usually contribute much information, only the affected transmissions are used to construct the score test, where "E(Aff)" is the number expected under the null hypothesis of no association. The unaffecteds are used to help impute missing parental alleles. Both "E(Aff)" and "SD(Aff)" are produced by a gene−dropping (with rejection sampling) algorithm. Using these simulated values, a *Z* statistic for over/undertransmission of each allele is calculated, and the asymptotic P−value for this statistic.

In this example, the overtransmission of the APOE*4 allele to Alzheimer's disease cases is the largest single contribution. Sib−pair combines the statistics from the alleles into a global test for which a simulation based P−value is produced. If the "plevel"=2, one obtains:

```
−−−−−−−−− Sibships used for RC−TDT −−−−−−−−−−−−−−−−−−−
Pedigree       Father          Mother      Aff Tot
F102              204 (2/3)    203 (2/2)   1   3  2/3
F106              101 (2/3)    102 (1/3)   4   5  2/3 1/3 3/3 2/3
F107              101 (2/3)    102 (2/3)   4   8  2/3 2/3 3/3 2/3
F118              101 (2/3)    102 (x/3)   2   3  3/3 3/3
F133              203 (1/3)    204 (2/3)   1   4  3/3
F133              202 (x/2)    201 (2/3)   1   2  2/3
F151              101 (x/2)    102 (2/3)   3   7  2/2 2/3 2/3
F163              101 (x/2)    102 (2/3)   4   6  2/3 2/2 2/3 2/3
F164              101 (x/2)    102 (2/3)   2   6  2/3 2/3
F175              201 (1/2)    202 (2/3)   3   6  1/2 2/2 2/2
```

```
F176              201 (2/3)    202 (x/3)   3   7  3/3 3/3 3/3
F197              101 (x/2)    102 (2/3)   3   6  2/3 2/2 2/3
F204              101 (2/3)    102 (x/3)   3   5  3/3 3/3 2/3


 Allele     Tr   E(Tr)  Cov(Tr)
------- ------ ------- ------------------------------------
    2        2    3.74    1.71
    3       28   34.45   −0.59    9.24
    4       38   29.80   −1.13   −8.65    9.78

 Parents genotyped   No. Fams  Useable  Aff Off
------------------   --------  -------  -------
 None                      24       12       33
 Father only                4        0        0
 Mother only               20        1        1
 Both parents              12        0        0
```

This is a tabulation of the contributing families, showing imputed and observed parental alleles, and the variance covariance matrix for the scores. Because this version of the FBAT is essentially a TDT with correct handling of missing parental genotypes, the verbose output labels the test "RC−TDT" (reconstructed (parental genotypes) TDT).

The "tdt" command performs the classical TDT of Spielman et al [1993], each allele versus all others, two multiallelic TDTs, and a genotypic TDT.

Here is an example using the TDT to fine map the Finnish late infantile neuronal ceroid lipofuscinosis gene (CLN5) in Finnish families segregating this rare Mendelian disorder (as per Figure 1 of Savukoski et al 1994).

```
>> include linclex.in
>> tdt oneperfam

−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
TDT for trait "oneperfam" v. all markers
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−

Marker     Typed  NParam Chi−square Asy P  Emp P  Iters
−−−−−−−−−− −−−−−− −−−−−− −−−−−−−−−− −−−−−− −−−−−− −−−−−−
D13S162        10      8       11.3 0.0068 0.0068      0 TDT−Best  *
D13S162        10      8       13.0 0.1118 0.0509    393 TDT−All   +
D13S162        10      7       19.8 0.0061 0.0061      0 TDT−Ewens *
D13S162        10      2       15.0 0.0006 0.0398    201 TDT−Gtp   **
D13S160        10      6       16.0 0.0002 0.0002      0 TDT−Best  **
D13S160        10      7       18.0 0.0120 0.0005   2001 TDT−All   **
D13S160        10      5       25.6 0.0001 0.0001      0 TDT−Ewens **
D13S160        10      1       20.6 0.0000 0.0100    201 TDT−Gtp   ***
D13S170         8     11        3.6 1.0000 1.0000      0 TDT−Best  .
D13S170         8     11       14.0 0.2330 0.0545    367 TDT−All   +
D13S170         8     10       14.2 0.1649 0.1649      0 TDT−Ewens .
D13S170         8      4       15.0 0.0047 0.2564     78 TDT−Gtp   *
```

The best results seem to be for D13S160.

```
>> keep oneperfam D13S160
>> set plevel 1
>> tdt oneperfam
```

So we will examine the detailed output.

```
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
TDT for trait "oneperfam" v. all markers
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−

Number of informative probands:  10

  − Allele by Allele TDT:"D13S160   " −
  Allele   Trans  Not Tr   TDT   P−value
  −−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
     1         2       4   0.7    0.6875
     2         0       6   6.0    0.0313
     3         0       3   3.0    0.2500
     4         0       4   4.0    0.1250
     5        16       0  16.0    0.0000
     6         0       1   1.0    1.0000

No. of alleles used  =    6
Bonferroni corr. 5%  =    0.010206
Bonferroni corr. 1%  =    0.002008
Bonferroni corr. 0.1%=    0.000200
```

The first table gives a TDT for each allele (versus all others). Heterozygous parents transmitted the "5" allele on 16 occasions, and it was never the nontransmitted allele. The fourth column is the TDT Chi−square, but the fifth column is the binomial exact P−value, but unadjusted for multiple testing (of 6 alleles).

In the summary table generated when `plevel`=0, the result for the best allele (allele 5) was given as "TDT−best", and the P−value **was** *Bonferroni corrected*.

```
  -------- Global Allelic TDT --------
   All 1   All 2    Tr=1    Tr=2   TDT
  -------------------------------------
     1       2         1       0   1.0
     1       4         1       0   1.0
     1       5         0       4   4.0
     2       5         0       5   5.0
     3       5         0       3   3.0
     4       5         0       3   3.0
     5       6         1       0   1.0

Allelic TDT Pearson chi−square=  18.0
            Degrees of freedom=   7
                       P−value=   0.0120
Empiric P−value (   2001 iter)=   0.0010

  Ewens allelic TDT chi−square=  25.6
            Degrees of freedom=   5
                       P−value=   0.0001
```

The next two tests are multiallelic TDTs. The table gives the pattern of transmission from each heterozygous parent to an affected child −− for example, there were 5 parents carrying the "2/5" genotype, and in each case the "5" allele was transmitted. The asymptotic P−value for this relatively sparse table is too conservative (P=0.012). By contrast, the empirical P−value, generated by permutation, is more impressive.

The Ewens test combines the six allelic TDT Chi−squares together into a (generally) more powerful multiallelic test.

```
  ----- Genotypic Transmission Test ------
       Genotype     Trans  Expected   Dev
  ---------------------------------------
          1/2          0       0.5   −0.7
          1/3          0       0.5   −0.7
          1/4          0       0.2   −0.4
          1/5          2       1.5    0.5
          1/6          0       0.2   −0.4
          2/3          0       0.2   −0.4
          2/4          0       1.0   −1.2
          2/5          0       1.2   −1.4
          3/5          0       0.8   −1.0
          4/5          0       0.8   −1.0
          5/5          7       1.8    2.6
          5/6          0       0.2   −0.4

Genotypic Transmission Chi−sq =  20.6
   Nominal degrees of freedom =   1
              Nominal P−value =   0.0000
      Equalled or exceeded by =   2/ 201 simulated values (0.0100)
Mean of simulated chi−squares =  10.8
```

Finally, the genotypic TDT is similar in flavour to the FBAT statistic in calculating the expected number of each genotype given the set of parental genotypes (mating types). It uses a CPG gene−dropping to assess significance. Because there are more genotypes than alleles, it is usually a less powerful test than an allelic TDT.

An alternative genotypic TDT is the loglinear model described by Schaid and Sommer [1993].

```
>>>> schaid oneperfam D13S160 5

------------------------------------------------
Schaid & Sommer analysis of trait "oneperfam"
------------------------------------------------
Versus allele   5 of marker D13S160

-------------------------------------------------------------
      Mating         Total Expected   5/5     5/−     −/−
-------------------------------------------------------------
  5/5   x   5/5          0      0.4     0       x       x
  5/5   x   5/−          0      2.0     0       0       x
  5/5   x   −/−          0      1.2     x       0       x
  5/−   x   5/−          8      2.5     7       1       0
  5/−   x   −/−          2      3.0     x       2       0
  −/−   x   −/−          0      8.1     x       x       0
-------------------------------------------------------------

Freq of   5 allele   =     0.050
N affected children  =    10

HWE Chi−square (2 df)=    30.73 (P=0.000)
Genotypic RR1 (f1)   = 11044.69 (95%CI=    0.00 to ********)
Genotypic RR2 (f2)   = ******** (95%CI=    0.00 to ********)
Attributable risk    =     1.00

CPG Chi−sq    (2 df) =    17.54 (P=0.000)
Genotypic RR1 (f1)   =   943.19 (95%CI=    0.00 to ********)
Genotypic RR2 (f2)   = 13204.69 (95%CI=    0.00 to ********)
Attributable risk    =     0.99
```

The Schaid and Sommer test has been implemented as a diallelic test, so it necessary to specify the marker, and the allele one is interested in testing. The "`schaid`" command actually presents results from two tests; one that is a "pure" TDT (the Conditional on Parental Genotypes "CPG Chi−square"), and a second that supplements this by testing for deviation in the mating type frequencies from Hardy−Weinberg Expectations (the "HWE Chi−square"). The "Expected" column in the table is the *expected number of that mating type*.

This procedure also presents the genotypic relative risks (and attributable risks), so that one can assess the mode of inheritance of the trait locus.

```
>>  hrr oneperfam

−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
Haplotype Relative Risk for trait "oneperfam" v. all markers
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−


  ---− HRR Analysis for "     D13S160   " −−−−−−−−−
    Allele   Affected    Control       Total    Dev
  −−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
     1        2 (.083)      4 (.190)       6   −1.1
     2        0 (.000)      6 (.286)       6   −2.9
     3        0 (.000)      4 (.190)       4   −2.5
     4        1 (.042)      5 (.238)       6   −2.0
     5       21 (.875)      1 (.048)      22    4.1
     6        0 (.000)      1 (.048)       1   −1.1
  −−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
   Total     24            21             45

       No. trait(+) marker(−) =     1
       No. trait(+) marker(+) =    12
   Contingency Pearson chi−sq =  32.5
   Nominal degrees of freedom =   5
              Nominal P−value =   0.0000
      Equalled or exceeded by =   1/90001 simulated values (0.0000)
 Mean (Var) simulated chi−sqs =   4.9 (   7.3)

>>  undrop
>>  set plevel 0
>>  hrr oneperfam

−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
Haplotype Relative Risk for trait "oneperfam" v. all markers
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−

Marker     Typed  NParam Chi−square Asy P  Emp P  Iters
---------- ------ ------ ---------- ------ ------ ------
D13S162        12      7       22.6 0.0020 0.0001  90001 HRR       ***
D13S160        12      5       32.5 0.0000 0.0000  90001 HRR       ***
D13S170         9     10       14.9 0.1362 0.0334    599 HRR       +
```

The haplotype relative risk test ("hrr") is not a commonly used test, as it sits (uneasily) between the TDT and a straight association test. In this test, the counts of the transmitted alleles are pooled and compared to the pooled counts of nontransmitted alleles, where pooling is across all the families. This means that genotypes from homozygous parents can be used (these are useless for the TDT).

If one is happy that there is no between family ethnic heterogeneity, then this approach will be more powerful than the TDT, and has the benefit that familial controls are being used. Sib−pair gene−drops for the P−value, so the test is appropriate for multigenerational or multiply affected pedigrees.

These results seem to suggest that CLN5 lies between D13S162 and D13S160 −− perhaps closer to D13S160. D13S170 is more distant.

Locus	Build 36.2 sequence position (bp)
D13S162	74874668

CLN5	76462796−76474653
D13S160	78076644
D13S170	80007129

Quantitative trait family based tests of association

There are two quantitative trait TDTs implemented in Sib−pair. The version obtained using "tdt" is the approach suggested by Gauderman [2003] which regresses trait value on genotype of the child, and includes the mating type (joint parental genotype) as a covariate. Returning to the Liu et al example:

```
>> tdt adres

-> tdt adres


-----------------------------------------------
TDT for trait "adres" v. all markers
-----------------------------------------------


  ------------ QTDT for "apoe      "-------------
    Allele   Allelic Mean    Stand Error   Count
  ---------------------------------------------
       2          -0.1049         0.1447       2
       3           0.0629         0.0589      32
       4          -0.0210         0.0330       8
  ---------------------------------------------
  Total           -0.0263         0.0715      42

 No. trait(+) marker(-)  =      0
 No. trait(+) marker(+)  =     21
 No. marker mating types =      6
 Allelic Mean Square     =      0.0094 (df=   2)
 Residual Standard Error =      0.0797 (df=  13)

 F-Statistic             =      0.7392
 Nominal P-value         =      0.4965
 Equalled or exceeded by =  223/  5001 simulated values (0.0446)
```

Association conditional on linkage

The Sib−pair "assoc" command can perform gene dropping conditional on *IBD* (Identity−by−Descent) at a marker, so it is possible to partition linkage and association in a "nonparametric" way. This is time−consuming, as *IBD* usually has to be simulated via MCMC (Monte−Carlo Markov Chain). Furthermore more simulations per marker need to be done.

Linkage Analysis

The Sib−pair commands for carrying out linkage analysis include:

asp	Affected sib pair linkage analysis
pen	Penrose sib pair linkage analysis
apm	Affected Pedigree member linkage analysis
sibpair	Regression based QTL linkage analysis
twopair	Two−point Haseman−Elston linkage analysis
qtlpair	Variance components QTL linkage analysis
lin	Sib pair intermarker linkage analysis

Intermarker linkage analysis

Keats and Elston suggested a simple method of estimating intermarker recombination distance based on the correlation in sib pair identity−by− descent sharing. This is not particularly powerful:

```
>>  lin

------------------------------------------------
Inter−marker sib pair linkage analysis
------------------------------------------------

Marker 1   Marker 2   Sibships Sibpairs  r(IBD) Recomb
---------- ---------- -------- --------  ------ -------
D13S162    D13S160          16       49   0.725   0.074 0.043−−0.126
D13S162    D13S170          13       48   0.523   0.138 0.081−−0.235
D13S160    D13S170          13       48   0.810   0.050 0.029−−0.087
```

The original Penrose sib pair method can be applied to codominant (or dominant) markers and/or categorical traits.

```
>> pen D13S162 D13S170
------------------------------------------------------------
Penrose Sib Pair Linkage Analysis for "D13S162" v. "D13S170"
------------------------------------------------------------

                    D13S170
D13S162    Concordant  Discordant
Concordant          6           3
Discordant          3          36

No. of sib pairs   =    48
No. of sibships    =    13

    No. complete observations =    48
    LR contingency chi−square =  13.72
           Degrees of freedom =   1
           Asymptotic P−value =   0.0002
                Cohen's Kappa =   0.5897
```

This method seems to capitalize on association as well as "pure" linkage.

Dichotomous trait nonparametric linkage analysis

The "apm" command is the main command for "NPL" (Nonparametric Linkage) type analysis. This implements single−marker linkage analysis.

```
>>  apm lincl

−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
APM for trait "     lincl" v. all markers
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−


Marker     NFams  NAff   Z−value    Asy P  Emp P  Iters
−−−−−−−−−− −−−−−− −−−−−− −−−−−−−−−− −−−−−− −−−−−− −−−−−−
D13S162         4     14        1.3 0.0982 0.1425    200 APM−IBS +
D13S162        12     14        2.8 0.0022 0.2889    200 GPM−IBS *
D13S160         4     14        1.3 0.0938 0.0590    200 APM−IBS +
D13S160        12     14        2.7 0.0030 0.1880    200 GPM−IBS *
D13S170         3     12        1.2 0.1202 0.0725    200 APM−IBS +
D13S170        11     12        4.3 0.0000 0.0001    200 GPM−IBS ***
```

The default analysis is an Identity−by−State (*IBS*) based affected pedigree member type analysis. With plevel=0, only the inverse−square−root allele−frequency weighted test scores are presented. There are two tests for each marker: one based on affected relative pair IBS sharing, and the (General Pedigree Method) GPM, which combines contributions from Aff−Aff, Aff−Una and Una−Una relative pairs. Both an asymptotic and a gene−dropping based empirical P−value are shown. By increasing the plevel=2:

```
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
APM for trait "     lincl" v. all markers
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−

−−−−−−−−−−− APM analysis for "D13S170" −−−−−−−−−−−−−−

Pedigree 1           E(Z)     Var(Z)         Z          T      MC−P
Aff−Aff
f(p) = 1              0.374      0.050      1.000      2.811 0.0350
f(p) = 1/sqrt(p)      1.063      0.435      1.642      0.878 0.1500
f(p) = 1/p            3.704     17.164      2.698     −0.243 0.5350
Aff−UnA
f(p) = 1              3.761      2.160      3.000     −0.518 0.3200
f(p) = 1/sqrt(p)     10.642     11.570      4.927     −1.680 0.0050
f(p) = 1/p           36.952    581.173      8.093     −1.197 0.0025
Aff−Aff v. Aff−UnA
f(p) = 1             −0.002      0.032      0.700      3.923 0.0025
f(p) = 1/sqrt(p)     −0.001      0.351      1.150      1.942 0.0400
f(p) = 1/p            0.009      9.414      1.888      0.613 0.1750
GPM
f(p) = 1             −0.009      0.010      0.350      3.647 0.0025
f(p) = 1/sqrt(p)     −0.037      0.098      0.847      2.828 0.0150
f(p) = 1/p           −0.189      2.106      2.760      2.032 0.0300

Affecteds: 3 4
Unaffectd: 7 6 5 2 1
    2 affecteds and     5 unaffecteds used
```

This prints the statistics for each family, and the contributing family members.

```
Overall statistics        T  NFam  Asy−P InvZ−P
Aff−Aff
f(p) = 1              3.704     3 0.0001 0.0058
f(p) = 1/sqrt(p)      1.269     3 0.1022 0.0667
f(p) = 1/p           −0.317     3 0.6244 0.4707
Aff−UnA
f(p) = 1              0.845     9 0.8008 0.9111
f(p) = 1/sqrt(p)     −0.419     9 0.3377 0.2562
f(p) = 1/p           −1.133     9 0.1286 0.0214
Aff−Aff v. Aff−UnA
f(p) = 1              4.963    11 0.0000 0.0243
f(p) = 1/sqrt(p)      2.563    11 0.0052 0.1105
f(p) = 1/p            0.873    11 0.1913 0.2913
GPM
f(p) = 1              4.169    11 0.0000 0.0012
f(p) = 1/sqrt(p)      4.090    11 0.0000 0.0001
f(p) = 1/p            2.760    11 0.0029 0.0017

Total of    12 affecteds and    46 unaffecteds used.
```

And finishes with the overall result. The "1/sqrt(p)" weighted score is usually suggested as the most trustworthy. These *IBS* based tests are very quick.

The equivalent analysis using Identity−by−Descent (*IBD*) is as easily performed.

```
>> apm lincl ibd

−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−
APM for trait "     lincl" v. all markers
−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−

NOTE:  Identity−by−descent based statistic used.


Marker     NFams  NAff   Z−value    Asy P  Emp P  Iters
−−−−−−−−−− −−−−−− −−−−−− −−−−−−−−−− −−−−−− −−−−−− −−−−−−
D13S162         4     15        2.2 0.0139 0.0327   5000 APM−IBD +
D13S162        13     15        2.2 0.0155 1.0000   5000 GPM−IBD +
D13S160         4     15        2.2 0.0141 0.0203   5000 APM−IBD +
D13S160        13     15        1.9 0.0302 1.0000   5000 GPM−IBD +
D13S170         3     12        1.3 0.0979 0.1260   5000 APM−IBD +
D13S170        11     12        1.0 0.1678 1.0000   5000 GPM−IBD .
```

One may be curious how these compare to results from other programs.

```
>> write locus merlin merlin.dat
>> write map merlin merlin.map
>> write merlin merlin.ped
>> $ merlin −−npl −−single
```

Doing the equivalent analysis in Merlin obtained:

```
Phenotype: lincl [ALL] (4 families)
=====================================================
            Pos   Zmean  pvalue    delta    LOD  pvalue
            min   −2.41     1.0   −0.577  −0.90     1.0
            max    2.99  0.0014    0.707   1.25   0.008
        D13S162    2.17    0.02    0.707   0.97    0.02
        D13S160    2.20   0.014    0.707   0.95    0.02
        D13S170    1.13    0.13    0.707   0.37    0.09
```

So, in this case the *IBS* based scores are far more impressive. This is because they pick up association as well as linkage, to a far greater extent than *IBD* based methods do.

In this instance, the Monte−Carlo based NPL (Nonparametric Linkage) method of Sib−pair is slower than Merlin. For larger pedigrees, time taken by MC (Monte−Carlo) based algorithms scales up slowly, compared to the Lander−Green algorithm.

```
>> include longqtex.in
```

A pedigree segregating the long QT syndrome has been used to test Monte Carlo Markov Chain linkage programs (see Jensen and Kong [1999]). The pedigree is "102 bits" in size, so requires an Elston−Stewart algorithm to estimate *IBD* deterministically.

```
>> show pedigrees
Pedigree    Size Fndrs  Gens Disjoint
---------- ----- ----- ----- --------
LQT           73    14     7

Total number of pedigrees  =        1
Number with only 1 member  =        0
Largest pedigree (members) =       73 (Pedigree LQT)
Deepest pedigree (genrtns) =        7 (Pedigree LQT)

Mean size of pedigrees     =     73.0
Mean pedigree depth        =      7.0
Mean size where >1 members =     73.0
Mean depth where >1 members=      7.0

>> set iter 5000

NOTE:  Number of MC iterations   5000

>> set timer on
[   0.00 s]

>> apm lqt ibd

-----------------------------------------------
APM for trait "       lqt" v. all markers
-----------------------------------------------

NOTE:  Identity-by-descent based statistic used.


Marker     NFams  NAff   Z-value    Asy P  Emp P  Iters
---------- ------ ------ ---------- ------ ------ ------
marker          1      5        4.1 0.0000 0.0014   5000 APM-IBD ***
marker          1      5        5.1 0.0000 0.0002   5000 GPM-IBD ***
[   5.94 s]

>> set iter 50000
>> set ple 1
>> apm lqt ibd

-----------------------------------------------
APM for trait "       lqt" v. all markers
-----------------------------------------------

NOTE:  Identity-by-descent based statistic used.

----------- APM analysis for "marker" --------------

Overall statistics        T  NFam  Asy-P InvZ-P

ibd-based Af-Af       4.094     1 0.0000 0.0021
ibd-based Af-Un       0.019     1 0.5076 0.5703
ibd-based GPM         5.166     1 0.0000 0.0001
Whit-Halp Score       6.833     1 0.0000 0.0020

Total of     5 affecteds and    68 unaffecteds used.
[  66.12 s]
```

Again, we can compare the results to those from other programs:

```
>> write locus morgan lqt.par lqt.ped
>> write morgan lqt.ped
```

The resulting parameter file will require a little editing in order to run *lm_ibdtests* from the MORGAN 2.8.2 suite of programs. The *Spairs* statistic is directly comparable:

```
[...]

 Trait data:

 Component 1:

    phenotype 2:
    27 28 39 41 56

[...]

 Now set 5 affected and 68 unaffected in component 1

[...]

 Starting M−sampler iteration 50000

 ******************************
 p Value for Normal Test for IBD
 ******************************

         pos(Haldane cM)
  locus     male  female   Spairs p−value  Srobdom p−value  Slambda p−value

 marker    0.000   0.000   4.0340  0.0000   9.0022  0.0000  −0.2347  0.5977

  system time:  0.030 sec
    user time:  139.530 sec
```

The *Slambda* test is designed to combine information from affected and unaffecteds, but in this case is most unimpressive. An upper bound on the "true" GPM result would be the lod score under a recessive model.

```
>> write locus linkage lqt.loc
>> write ppd lqt.ppd
```

After changing the penetrances in the resulting locus file from a dominant to a recessive model,

```
    2  0  0  5  0
0 0.0 0.0 0
 1 2
1     2 # lqt                  #
0.990000 0.010000
    1
0.000000 0.000000 1.000000
3     4 # marker               #
 0.4221 0.0556 0.2556 0.2667
0 0
 .0
1 0.05 0.4
```

SUPERLINK (V 1.6) says the lod score under that model at *c*=0 is 6.83 (expressed as a Z−score, this is 5.61). The affecteds−only analysis gives lod=3.25, equivalently Z=3.87.

SIMWALK2 gives a parametric lod score close to the above under the same model, but gives quite different nonparametric results:

```
>> write locus mendel lqt.lom trait
>> write mendel lqt.pem trait
>> write map mendel lqt.map
```

The `trait` modifier means that a dichotomous trait will appear as a diallelic locus rather than a *factor* in the locus and pedigree files.

```
    Results of the Non−Parametric Linkage (NPL) Analysis from SimWalk2 2.91

 The results for the pedigree named: LQT                                  ##
 which is pedigree number:           001                                  ##
 This run has the integer label:     03                                   ##

 The number of individuals is:       73
 The number of affecteds is:          5
[...]
                              BLOCKS    MAX−TREE  ENTROPY   NPL_PAIR  NPL_ALL
                              RECESSIVE DOMINANT  STATISTIC ADDITIVE  ADDITIVE
                              ~~~~~~~~~ ~~~~~~~~~ ~~~~~~~~~ ~~~~~~~~~ ~~~~~~~~
 RUN  PED  PEDIGREE  MARKER   P−VALUE   P−VALUE   P−VALUE   P−VALUE   P−VALUE
 NUM  NUM    NAME     NAME    −Log(P)   −Log(P)   −Log(P)   −Log(P)   −Log(P)
 ____________________________________________________________________________

 03   001   LQT     marker     0.0386    0.0308    0.0156    0.0173    0.0143
                               1.4129    1.5117    1.8061    1.7630    1.8432
____________________________________________________________________________
```

Quantitative trait linkage analysis

Sib−pair provides regression based linkage analysis for full and half sib pairs, and variance components linkage analysis for arbitrary pedigrees.

Using a well known hypercholesterolemia pedigree [Williams et al 1986], we can test some of these commands:

```
>> include williamsex.in
>> set locus logChol qua
>> logChol=log(adjChol)
>> his adjChol
>> his logChol
>> des logChol
>> var logChol
```

Obviously log transforming serum cholesterol level makes the trait closer to Gaussian, changing the Filliben correlation from 0.94 to 0.98 (this is the correlation between the observed values and where they would lie if the trait was Gaussian based on their rank in the distribution).

Because the dataset is small, the pattern of relative pair correlations is quite variable, and the variance components analysis estimates the heritability as zero.

```
>> set plevel 1
>> sib logChol sim

------------------------------------------------------
Sham S+D H−E for trait "logChol" v. all markers
------------------------------------------------------

----------------------------------------------
 H−E analysis for "logChol" v. "ldl"
----------------------------------------------

Trait mean (nonfo) =     5.1250 (SD=    0.6868)
Sibling r          = 0.039 (   44 pairs)
Half−sib r         = −.038 (    1 pairs)
Working half−sib r = 0.020

No. full−sib pairs =    44 (in    15 sibships)
No. half−sib pairs =     1
Mean full−sib ibd  = 0.481
Intercept (f−s)    =     0.0000 (ase=    0.0000)
Slope              =     2.1835 (ase=    0.4510)
t value            =     4.8410 (df=  22, P=0.0000)

Equalled or exceeded by =  20/  158 simulated values (0.1266)
Mean (SD) simulated Beta=      0.4607 (      2.8133)

Score test (f−s)   =     3.9591 (P=0.0000)
Robust Disc Pair t =     2.7786 (P=0.0027)
```

There are results for three linkage tests here. The first test is the Sham and Purcell [2001] regression based on weighted contributions from within−pair trait sums and differences. This requires a working estimate of the sibling trait correlation (to calculate weights). It is highly significant (t=4.84, df=22). The modifier "`sim`" keyword added to the "`sibpair`" command asked for a gene−dropping P−value, which is quite different.

The last two lines give results from a score test for linkage and the updated Robust Discordant Pairs test both from Szatkiewicz and Feingold [2004]. The latter is useful for selected samples, such as those recruited under an EDAC design. These are again significant, although a simulated P−value is not currently calculated for them, so we cannot test quite how robust they are.

```
>> set plevel 1
>> qtl logChol
-----------------------------------------------------------
VC linkage analysis for trait "logChol" v. all markers
-----------------------------------------------------------

-----------------------------------------------------
VC linkage analysis for "logChol" v "ldl"
-----------------------------------------------------

Number of sibships         =    23
Number of observations     =    45
Trait mean                 =     5.292256
Additive genetic variance  =     0.000000 (  0.0%)
QTL genetic variance       =     0.450176 ( 93.7%)
Environmental variance     =     0.030146 (  6.3%)
Linkage chi−square (lod)   =     8.89     ( 1.93)
Total function evaluations =   536
```

The default variance components linkage analysis is based on sibships only, so it should give results consistent with the regression based approaches.

```
>> set ple 0
>> qtl logChol full

-----------------------------------------------------------
VC linkage analysis for trait "logChol" v. all markers
-----------------------------------------------------------

Marker     NFams  NPheno lod score  Asy P  Emp P  Iters
---------- ------ ------ ---------- ------ ------ ------
ldl             1     46        3.9 0.0000 1.0000      0 VC  ***

>> set ple 1
>> qtl logChol full

-------------------------------------------------
Variance components analysis for "logChol"
-------------------------------------------------
Random: A+Q{ldl}

Number of families         =     1
Number of observations     =    46
Trait mean (intercept)     =     5.039116

Additive genetic variance  =     0.000000 (  0.0%)
QTL genetic variance       =     0.249035 ( 68.8%)
Environmental variance     =     0.113122 ( 31.2%)
Model loglikelihood        =     6.497649

Chi−square testing VQ=0    =    17.97     (df=1, P=0.000)

Chi−square testing VG=0    =    17.97     (df=2, P=0.000)
Total function evaluations =   340
```

With the "full" modifier, "qtl" calls a Monte−Carlo Markov Chain based routine to estimate *IBD* for all phenotyped individuals, and carries out a variance components linkage analysis using the entire pedigree. Sib−pair also generates a multipoint estimate of *IBD* if the markers are close enough together (default is 0.1 cM) under the assumption there is negligible intermarker recombination by combining adjacent single−point *IBD* estimates for each pair of relatives.

Generalized Linear Mixed Models

The "fpm" is a more experimental command, and results should be checked carefully. It offers fitting of finite polygenic models, single major gene and mixed segregation models, ordinary variance components (also called linear mixed) models, and *generalized linear mixed models*

A Generalized Linear Mixed Model (GLMM) is a *generalized linear model* that includes random effects (such as unmeasured genes). A generalized linear model is the extension of linear regression to non−Gaussian distributions, including the binomial distribution (logistic regression), the Poisson distribution (suitable for analysis of counts or survival times), and others from the exponential family. Poisson regression can be used to fit the semiparametric Cox proportional hazards model (commonly used to analyse survival or age−at−onset data) using a piecewise baseline hazard, and modified to perform Weibull distribution based "parametric" survival regressions. Therefore these are very flexible models that allow analysis of "difficult" traits.

Sib−pair uses Monte−Carlo Markov Chain (MCMC) algorithms to fit GLMMs to pedigree data. MCMC is a simulation based method of fitting statistical models, that has been applied to genetic problems in the programs LOKI, MORGAN and SIMWALK2 (among others). A disadvantage of the MCMC approach is that it can be slow and convergence to the correct solution is not assured.

In teratogeneticity experiments on experimental animals, it is necessary to adjust for litter effects − genetic or maternal modifiers of teratogen action. Slaton et al [2000] present an analysis of data on the developmental toxicity of boric acid in mice, where both the proportion of offspring affected and the sibship binary correlation increase with increasing dose of boric acid (gene by environment interaction). To model this effect, I have placed all sibships where the dam received the same dose in to the same "family environmental" group.

```
>> fpm nonviable nqtl 0 c s cov dose
```

The "nqtl 0" modifier of the "fpm" command forces the fitting of a polygenic/variance components type model. The "c s" keywords fit a common environmental random effect (dose) and a sibship/litter effect. The "cov dose" keywords added a fixed−effects model of dose of boric acid on mean risk of nonviability.

```
------------------------------------------------
Finite Polygenic Model analysis for "nonviable"
------------------------------------------------

Number of families         =       4
Number of sibships         =     107
Number of observations     =    1297
Burn−in MCMC iterations    =    1000
Evaluated MCMC iterations  =   10000
Number of MCMC chains      =       4
Metropolis sampler         = Sliced
Model type                 = Binomial
```

```
Link type                  = Logit
Fixed Effects              = nonviable ~ mu + dose
Random Effects             = VC VS VE

Global trait mean          =     0.101773
Global trait variance      =     0.091416

Number of simulated QTLs   =     0
```

The first part of the output summarizes the model (the "families" are actually dosage groups). In this run, I increased the value of "`iterations`" to 1000, which "`fpm`" automatically multiplies by 10. In addition, I increased the number of MCMC chains to 4. This in fact replicates each family four times in the analysis, and calculates average results over the replicates, so the estimation of the unobserved random effects is more precise. For this job, increasing the number of chains greatly improves the accuracy of estimation.

```
Intercept                  =    −3.217950
Family environmental var   =     0.177838 (  0.0%)
Maternal effect variance   =     1.066121 (  0.0%)
Environmental variance     =     0.037580 (100.0%)
Modal model loglikelihood  =  −509.778
Mean model loglikelihood   =  −510.546
C.V. Loglikelihood         =     1.39%

Summarized run as    99 batches of size        101 :

Parameter             Mean        Mode          SD        Z−value     MC−SE   Fixed
----------------  ----------   ----------   ----------   ----------  -------- -----
mu                  −3.2179      −3.1593       0.1982      16.2336    0.0281
VC                   0.1778       0.1507       0.1175       1.5135    0.0085
VS                   1.0661       1.0295       0.1886       5.6534    0.0226
VE                   0.0376       0.0386       0.0069       5.4682    0.0010
sdC                  0.4008       0.3901       0.1311       3.0580    0.0098
sdS                  1.0286       1.0111       0.0898      11.4586    0.0106
sdE                  0.1931       0.1932       0.0176      10.9524    0.0025
dose                 3.0725       2.7731       0.7714       3.9831    0.1220
Realized VC          0.1318       0.1265       0.0667       1.9767    0.0056
Realized VS          1.0615       1.0277       0.1724       6.1574    0.0211
Realized VE          0.0764       0.0765       0.0006     124.0765    0.0000
VE(F+R)/VE(F)        0.8459       0.8418       0.0110      76.5620    0.0011
```

```
Proposal               N   Accepted
------------ ------------- --------
Genotype          13447900    0.594 (M)
mu                   11527    0.191 (S)
VC                   10747    0.205 (S)
VS                   11497    0.190 (S)
dose                 11375    0.192 (S)

NOTE:  For ordinary Metropolis sampling (M), the proposal
       acceptance rate is optimally 0.2−0.6.  The slice
       sampler (S) is more robust, but slower.
```

The first section summarises the model parameters and model likelihood. These estimates are repeated in the tabulation that follows: the mean and mode of the simulated values along with the standard deviation of the simulated values −− which is equivalent to the standard error from a standard analytic approach.

The mean and standard error are used to give the next column, which gives a Z−score testing the hypothesis that the parameter equals zero. This can compared to the standard Gaussian distribution to give a P−value.

The final column of values is the estimated Monte−Carlo standard error for each parameter estimate (via the batch means method). Because we are fitting the model via MCMC simulation, aside from the usual statistical sources of error, there is additional noise due to the simulation process itself. This can be made smaller by averaging over more simulations (increasing `iterations`), but this of course takes more time. The largest MC−SE value is seen for the fixed effect of dose, so this parameter estimate will show a 4% (0.12/3.07) error in either direction on rerunning the model.

The results confirm that boric acid is a teratogen (regression coefficient for dose=3.07, Z=3.13, P=0.002), but conclude that under a GLMM with Gaussian−distributed random effects there is no significant effect of dose group on familial correlation (VC=0.1778, Z=1.51, P=0.13). There *is* a significant litter effect, so a naive logistic regression, ignoring the relatedness of the mice gives an inappropriately high Z statistic of 5.52.

For this example, we can obtain analytic results for the same model using other programs (that are considerably faster for this model as well). We prepare the data to be read into the **R** statistical package [R Development Core Team 2006], by using the "`write csv`" file command:

```
>> write csv boric.csv
```

The *lmer* function in R gives (after 1−2 seconds):

```
> boric <- read.csv("boric.csv")
> names(boric)[1] <- "dose.group"
> names(boric)[4] <- "litter"
> library(lme4)
Loading required package: Matrix
Loading required package: lattice
> lmer(nonviable ~ dose + (1|litter) + (1|dose.group), family=binomial, data=boric)
```

```
Random effects:
 Groups     Name        Variance Std.Dev.
 litter     (Intercept) 1.027175 1.01350
 dose.group (Intercept) 0.016316 0.12773
number of obs: 1297, groups: litter, 107; dose.group, 4

Estimated scale (compare to  1 )  0.883114

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  −3.1986     0.2596 −12.323  < 2e−16 ***
dose          3.2647     1.0515   3.105  0.00190 **
```

Unfortunately, *lmer* does not give standard errors for the random effects variances. Another R package (*glmmML*) will give standard errors for a single random effect, so fitting the model without the dosage group random effect, we obtain,

Parameter name	Sib−pair estimate (SE)	glmmML estimate (SE)	lmer estimate (SE)
Dose (fixed effect)	3.250 (0.936)	3.299 (0.974)	3.299 (0.972)
Sibship SD	1.039 (0.159)	1.029 (0.156)	1.029

The Monte−Carlo error for dose is relatively large, so the standard error reported here may vary a bit if we rerun the example (with different random number generator seeds).

You may be curious what the usual geneticists' model for dichotomous data gives. To run the multifactorial threshold model using SOLAR:

```
>> write solar boric.ped
>> write solar boric.phe phe
```

Sib−pair writes both an MZ twin ID (if "set twin <indicator>" has been issued) and a household ID (hhid) to the Solar pedigree file. And:

```
> load pedigree boric.ped
> load phenotypes boric.phe
> trait nonviable
> covariate dose
> house
> polygenic −screen
```

This takes some hours to run.

Model	LogL	h^2	c^2	dose Beta (SE)
E	−411.58	−	−	−
CE	−411.44	−	0.00	−
AE	−393.64	0.38 (0.088)	−	1.477 (0.397)
ACE	−393.64	0.38 (0.088)	0.00	1.477 (0.397)
Dropping Dose	−399.72	0.38	0.00	−

The LRTS for a dose fixed effect is 12.15 (df=1) under the SOLAR multifactorial threshold model, while it was 10.82 using *lmer*'s binomial GLMM.

References

- Gauderman WJ [2003]. Candidate gene association analysis for a quantitative trait, using parent−offspring trios. *Genet Epidemiol* **25**: 327−338.
- Jensen CS, Kong A [1999]. Blocking Gibbs sampling for linkage analysis in large pedigrees with many loops. *Am J Hum Genet* **65**: 885−901.
- Lawler SD, Sandler M [1954]. Data on linkage in man: elliptocytosis and blood groups. IV. Families 5, 6 and 7. *Ann Eugen* 18: 328−34.
- Morton NE [1956]. The detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type *Am J Hum Genet* **8**: 80−96.
- Liu L, Forsell C, Lilius L, Axelman K, Corder EH, Lannfelt L [1996]. Allelic association but only weak evidence for linkage to the apolipoprotein E locus in late−onset Swedish Alzheimer families. *Am J Med Genet* **67**: 306−311.
- R Development Core Team [2007]. R: A Language and Environment for Statistical Computing. Version 2.5.0 [Computer Program]. Vienna: R Foundation for Statistical Computing. http://www.R−project.org
- Savukoski M, Kestila M, Williams R et al [1994]. Defined chromosomal assignment of CLN5 demonstrates that at least four genetic loci are involved in the pathogenesis of human ceroid lipofuscinoses. *Am J Hum Genet* **55**: 695−701.
- Schaid DJ, Sommer SS [1993]: Genotype relative risks: methods for design and analysis of candidate−gene association studies. *Am J Hum Genet* **53**: 1114−1126.
- Schellenberg GD, Bird TD, Wijsman EM et al [1992]. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* **258**: 668−671.
- Slaton SL, Piegorsch WW, Durham SD [2000]. Estimation and Testing with Overdispersed Proportions Using the Beta−Logistic Regression Model of Heckman and Willis. *Biometrics* **56**: 125−133.
- Szatkiewicz JP, Feingold E [2004]. A powerful and robust new linkage statistic for discordant sibling pairs. *Am J Hum Genet* **75**: 906−909.
- Williams RR, Hasstedt SJ, Wilson DE, Ash KO, Yanowitz FF, Reiber GE, Kuida H [1986]. Evidence that men with familial hypercholesterolemia can avoid early coronary death. An analysis of 77 gene carriers in four Utah pedigrees. *JAMA* **255**: 219−224.