SAS Publishing

SAS/Genetics™ 9.1

User's Guide

The Power to Know®

The correct bibliographic citation for this manual is as follows: SAS Institute Inc. 2004. *SAS/Genetics™ 9.1 User's Guide*. Cary, NC: SAS Institute Inc.

SAS/Genetics™ 9.1 User's Guide

ISBN 1-59047-233-0

SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513.

1st printing, January 2004

Contents

Acknowledgments

Credits

Documentation

Writing	Wendy Czika and Xiang Yu
Editing	Virginia Clark and Rob Pratt
Technical Review	Yujun Shao and Russell D. Wolfinger
Documentation Production	Tim Arnold

Software

The procedures in SAS/Genetics software were implemented by members of the Analytical Solutions Division. In the following list, the names of the developers currently supporting the procedure are listed first. Other developers previously worked on the procedure.

ALLELE	Wendy Czika
CASECONTROL	Wendy Czika
FAMILY	Wendy Czika
HAPLOTYPE	Xiang Yu
HTSNP	Xiang Yu
INBREED	Anthony Baiching An, Meltem Narter
PSMOOTH	Wendy Czika
TPLOT Macro	Wendy Czika
TPLOT Results (Frame)	Art Barnes
TPLOT Results (SCL)	Susan E. Haller

Support Groups

Quality Assurance	Jack J. Berry, Ming-Chung Chan, Kelly M. Graham, Gerardo I. Hurtado, Yujun Shao, and Audrey Ventura
Technical Support	Rob Agnelli and Kathleen Kiernan

Acknowledgments

Many people make significant and continuing contributions to the development of SAS software products. The following are some of those who have contributed significant amounts of their time to help us make improvements to SAS/Genetics software. This includes research and consulting, testing, or reviewing documentation. We are grateful for the involvement of these members of the statistical community and the many others who are not mentioned here for their feedback, suggestions, and consulting.

Peter Boyd	GlaxoSmithKline
Margaret G. Ehm	GlaxoSmithKline
Greg Gibson	North Carolina State University
Shizue Izumi	Radiation Effects Research Foundation
Bret Musser	Merck
Dahlia Nielsen	North Carolina State University
Bruce S. Weir	North Carolina State University
Dmitri Zaykin	GlaxoSmithKline

The simulated GAW12 data used in this book are supported by NIGMS grant GM31575.

The final responsibility for the SAS System lies with SAS alone. We hope that you will always let us know your opinions about the SAS System and its documentation. It is through your participation that SAS Software is continuously improved.

Please send your comments to **suggest@sas.com**.

What’s New in SAS/Genetics 9 and 9.1

Contents

What's New in SAS/Genetics 9 and 9.1

Overview

SAS/Genetics includes several new procedures:

- the experimental HTSNP procedure for selecting a subset of SNPs that identify groups of haplotypes that minimize within-group diversity 9.1
- the INBREED procedure for estimating covariance and/or inbreeding coefficients for pedigrees 9.1

New features have been added to the SAS/Genetics procedures: 9.1

- ALLELE
- CASECONTROL
- FAMILY
- HAPLOTYPE
- PSMOOTH

including options that accommodate new data formats.

Accommodating New Data Formats

There are several new options available for analyzing data in different formats. 9.1 The GENOCOL and DELIMITER= options have been added to four procedures: ALLELE, CASECONTROL, FAMILY, and HAPLOTYPE. The GENOCOL option enables you to use columns containing marker genotypes instead of a pair of columns containing the two alleles that comprise the genotype. You can specify the delimiter that is used to separate the two alleles with the DELIMITER= option. In addition, the experimental options TALL, MARKER=, and INDIV= can be used collectively for data in a “tall-skinny” format in the ALLELE, CASECONTROL, and HAPLOTYPE procedures. Data sets in this format contain a marker identifier and individual identifier, along with one variable containing the marker genotypes or two columns containing marker alleles. See the individual procedures’ Syntax sections for more details about these new options.

ALLELE Procedure

9.1 The new options ALLELEMIN=, GENOMIN=, and HAPLOMIN= enable you to specify the minimum estimated frequency for an allele, genotype, or haplotype, respectively, to be included in its corresponding ODS table. By default, any allele, genotype, or haplotype that occurs at least once in the sample is included in the respective table. These options can be used to reduce the size of the ODS tables, or alternatively, the GENOMIN= or HAPLOMIN= options can be set to 0 to view all possible genotypes or haplotypes, not just those that are observed.

CASECONTROL Procedure

9.1 The new NULLSNPS= option enables you to specify SNPs to be used in calculating the variance inflation factor for genomic control. By default, if VIF is specified, the variables in the VAR statement are used, but this new option provides a way of using particular SNPs, separate from those being tested for association and which are assumed to have no association with the TRAIT variable, for genomic control (Bacanu, Devlin, and Roeder 2000).

9.1 You can request that approximations of exact p-values for the case-control tests be reported in place of the asymptotic chi-square p-values (Westfall and Young 1993). The new PERMS= option indicates the number of permutations to be used for a Monte Carlo estimate of each exact p-value, and the random seed can be provided in the new SEED= option.

9.1 The OUTSTAT= data set includes two new columns: NumTrait1 and NumTrait2, where the values 1 and 2 are replaced by the two values of the TRAIT variable. These columns contain the number of genotyped individuals with each trait value for each marker.

FAMILY Procedure

9.1 The new "Family Summary" ODS table displays information about each family in the data set at each of the markers. This includes the number of parents genotyped, the number of affected children, the number of unaffected children, as well as an error code indicating what type of, if any, Mendelian inconsistencies occur in a nuclear family's genotypes at each marker. The new SHOWALL option can be used to display this information for all families at each marker. By default, only those families with a genotype error are included in the table for the marker(s) where the error occurs.

9.1 The new "Description of Error Codes" ODS table provides descriptions of the numerical error codes used in the "Family Summary" table.

9.1 Approximations of exact p-values can now be requested in place of the asymptotic chi-square p-values for the TDT, S-TDT, SDT, and combined S-TDT and SDT using the PERMS= option. The number specified indicates the number of permutations to be used in the Monte Carlo procedure for estimating exact p-values. You can provide the random seed used for the permutations in the new SEED= option.

The multiallelic SDT and multiallelic combined SDT/TDT are now implemented as described by Czika and Berry (2002). 9.1

HAPLOTYPE Procedure

The EST=EM | STEPEM option enables you to specify whether you would like haplotype frequencies to be estimated using the original EM algorithm or the new stepwise EM algorithm (Clayton 2002). When EST=STEPEM is specified, a cutoff to be used for trimming the set of haplotypes before adding an additional locus can be given in the new STEPTRIM= option. 9.1

The ID statement enables variables from the input data set to be included in the OUT= data set created by PROC HAPLOTYPE in addition to or instead of the _ID_ variable, a unique numeric identifier assigned to each individual by the procedure. 9.1

HTSNP Procedure

The experimental HTSNP procedure implements search algorithms for identifying a subset of SNPs called *haplotype tag SNPs (htSNPs)* (Johnson et al. 2001) that capture much of the linkage disequilibrium and haplotype diversity among common haplotypes. 9.1

INBREED Procedure

The INBREED procedure is now included in SAS/Genetics in addition to SAS/STAT where it originated. This procedure calculates the covariance or inbreeding coefficients for pedigrees either by treating the population as a single generation or by performing separate analyses on each generation. You can also opt to have inbreeding and covariance coefficients averaged within each gender category. 9.1

PSMOOTH Procedure

The new option TPM implements the truncated product method (Zaykin et al. 2002) for smoothing p-values over windows of markers. The TAU= option, also new, can be used in conjunction with the TPM option to specify the value of τ at which p-values are truncated. 9.1

References

Bacanu, S-A., Devlin, B., and Roeder, K. (2000), "The Power of Genomic Control," *American Journal of Human Genetics,* 66, 1933–1944.

Clayton, D. (2002), "Choosing a Set of Haplotype Tagging SNPs from a Larger Set of Diallelic Loci," [http://www-gene.cimr.cam.ac.uk/clayton/software/stata/htSNP/htsnp.pdf].

Czika, W. and Berry, J.J. (2002), "Using All Alleles in the Multiallelic Versions of the SDT and Combined SDT/TDT," *American Journal of Human Genetics,* 71, 1235–1236.

Johnson, G.C.L. et al. (2001), "Haplotype Tagging for the Identification of Common Disease Genes," *Nature Genetics,* 29, 233–237.

Westfall, P.H. and Young, S.S. (1993), *Resampling-based Multiple Testing,* New York: John Wiley & Sons, Inc.

Zaykin, D.V., Zhivotovsky, L.A., Westfall, P.H., and Weir, B.S. (2002), "Truncated Product Method for Combining P-values," *Genetic Epidemiology,* 22, 170–185.

Chapter 1
Introduction

Chapter Contents

Chapter 1
Introduction

Overview of SAS/Genetics Software

Statistical analyses of genetic data are now central to medicine, agriculture, evolutionary biology, and forensic science. The inherent variation in genetic data, together with the substantial increase in the scale of genetic data following the human genome project, has created a need for reliable computer software to perform these analyses. The procedures offered by SAS/Genetics and described here represent an initial response of SAS Institute to this need.

Although many of the statistical techniques used in the new procedures are standard, others have had to be developed to reflect the genetic nature of the data. All the procedures are designed to operate on data sets that have a familiar structure to geneticists, and that mirror those used in existing software. The syntax for these genetic analyses follows that familiar to SAS users, and the output can be tabular or graphical. The objective of the procedures is to bring the full power of SAS analyses to bear on the characterization of fundamental genetic parameters, and most importantly on the detection of associations between genetic markers and disease status.

Most of the analyses in SAS/Genetics are concerned with detecting patterns of covariation in genetic marker data. These data generally consist of pairs of discrete categories; this pairing derives from the underlying biology, namely the fact that complex organisms have pairs of chromosomes. Each marker refers to the genetic status of a *locus,* each marker type is called an *allele,* and each pair of alleles in an individual is called a *genotype.* A set of alleles present on a single chromosome is called a *haplotype.* Genetic markers may be single nucleotide polymorphisms (SNPs), which are sites in the DNA where the nucleotide varies among individuals, usually with only two alleles possible; microsatellites, which are simple sequence repeats that generate usually between 2 and 20 categories; and other classes of DNA variation.

Two of the procedures in SAS/Genetics are concerned solely with the analysis of genetic marker data. The ALLELE procedure calculates descriptive statistics such as the frequency and variance of alleles and genotypes, as well as estimating measures of marker informativeness, and testing whether genotype frequencies are consistent with Hardy-Weinberg equilibrium (HWE). This procedure also supports three methods for calculation of the degree and significance of *linkage disequilibrium* (LD) among markers at pairs of loci, where LD refers to the propensity of alleles to cosegregate. The HAPLOTYPE procedure is used to infer the most likely multilocus haplotype frequencies in a set of genotypes. Since genetic markers are usually measured independently of one another, there is no direct way to determine which two alleles were on the same chromosome. The algorithm implemented in this procedure converges on the haplotype frequencies that have the highest probability of generating the observed genotypes.

Many genetic data sets are now used to study the relationship between genetic markers and complex phenotypes, particularly disease susceptibility. In general terms, traits can be measured as continuous variables (for example, weight or serum glucose concentration), as discrete numerical categories (for example, meristic measures or psychological class), or as affected/unaffected indicator variables. The two procedures CASECONTROL and FAMILY both take simple dichotomous indicators of disease status and use standard genetic algorithms to compute statistics of association between these indicators and the genetic markers. The CASECONTROL procedure is designed to contrast allele and genotype frequencies between affected and unaffected populations, using three types of chi-square tests and options for controlling correlation of allele frequencies among members of the same subpopulation. Significant associations may indicate that the marker is linked to a locus that contributes to disease susceptibility, though population structure in conjunction with environmental or cultural variables can also lead to associations, and the statistical results must be interpreted with caution. The FAMILY procedure employs several transmission/disequilibrium tests of nonrandom association between disease status and linkage to markers transmitted from heterozygous parents to affected offspring (TDT) or pairs of affected and unaffected siblings (S-TDT and SDT). A joint analysis known as the reconstruction-combined TDT (RC-TDT) can also accommodate missing parental genotypes and families lacking unaffected children under some circumstances.

The output of these procedures can be further explored by using the PSMOOTH procedure to adjust p-values from association tests performed on large numbers of markers obtained in a genome scan, or by creating a graphical representation of the procedures' output, namely p-values from tests for LD, HWE, and marker-disease associations, using the %TPLOT macro.

About This Book

Since SAS/Genetics software is a part of the SAS System, this book assumes that you are familiar with base SAS software and with the books *SAS Language Reference: Dictionary*, *SAS Language Reference: Concepts,* and the *SAS Procedures Guide*. It also assumes that you are familiar with basic SAS System concepts such as creating SAS data sets with the DATA step and manipulating SAS data sets with the procedures in base SAS software (for example, the PRINT and SORT procedures).

Chapter Organization

This book is organized as follows.

Chapter 1, this chapter, provides an overview of SAS/Genetics software and summarizes related information, products, and services. The next five chapters describe the SAS procedures that make up SAS/Genetics software. These chapters appear in alphabetical order by procedure name. They are followed by a chapter documenting a SAS macro provided with SAS/Genetics software.

The chapters documenting the SAS/Genetics procedures are organized as follows:

- The *Overview* section provides a brief description of the analysis provided by the procedure.
- The *Getting Started* section provides a quick introduction to the procedure through a simple example.
- The *Syntax* section describes the SAS statements and options that control the procedure.
- The *Details* section discusses methodology and miscellaneous details.
- The *Examples* section contains examples using the procedure.
- The *References* section contains references for the methodology and examples for the procedure.

Typographical Conventions

This book uses several type styles for presenting information. The following list explains the meaning of the typographical conventions used in this book:

roman — is the standard type style used for most text.

UPPERCASE ROMAN — is used for SAS statements, options, and other SAS language elements when they appear in the text. However, you can enter these elements in your own SAS programs in lowercase, uppercase, or a mixture of the two.

UPPERCASE BOLD — is used in the “Syntax” sections’ initial lists of SAS statements and options.

oblique — is used for user-supplied values for options in the syntax definitions. In the text, these values are written in *italic*.

helvetica — is used for the names of variables and data sets when they appear in the text.

bold — is used to refer to matrices and vectors.

italic — is used for terms that are defined in the text, for emphasis, and for references to publications.

`monospace` — is used for example code. In most cases, this book uses lowercase type for SAS code.

Options Used in Examples

Output of Examples

For each example, the procedure output is numbered consecutively starting with 1, and each output is given a title. Each page of output produced by a procedure is enclosed in a box. Most of the output shown in this book is produced with the following SAS System options:

```
options linesize=80 pagesize=200 nonumber nodate;
```

In some cases, if you run the examples, you will get slightly different output depending on the SAS system options you use and the precision used for floating-point calculations by your computer. This does not indicate a problem with the software. In all situations, any differences should be very small.

Graphics Options

The examples that contain graphical output are created with a specific set of options and symbol statements. The code you see in the examples creates the color graphics that appear in the online (CD) version of this book. A slightly different set of options and statements is used to create the black-and-white graphics that appear in the printed version of the book.

If you run the examples, you may get slightly different results. This may occur because not all graphic options for color devices translate directly to black-and-white output formats. For complete information on SAS/GRAPH software and graphics options, refer to *SAS/GRAPH Software: Reference*.

The following GOPTIONS statement is used to create the online (color) version of the graphic output.

```
filename GSASFILE  '<file-specification>';

goptions gsfname=GSASFILE   gsfmode =replace
         fileonly
         transparency       dev     = gif
         ftext    = swiss   lfactor = 1
         htext    = 4.0pct  htitle  = 4.5pct
         hsize    = 5.625in vsize   = 3.5in
         noborder           cback   = white
         horigin = 0in      vorigin = 0in ;
```

The following GOPTIONS statement is used to create the black-and-white version of the graphic output, which appears in the printed version of the manual.

```
filename GSASFILE  '<file-specification>';

goptions gsfname=GSASFILE   gsfmode =replace
         gaccess = sasgaedt fileonly
         dev     = pslepsf
         ftext    = swiss   lfactor = 1
         htext    = 3.0pct  htitle  = 3.5pct
         hsize    = 5.625in vsize   = 3.5in
         border             cback   = white
         horigin = 0in      vorigin = 0in ;
```

In most of the online examples, the plot symbols are specified as follows:

```
symbol1 value=dot color=white height=3.5pct;
```

The SYMBOL*n* statements used in online examples order the symbol colors as follows: white, yellow, cyan, green, orange, blue, and black.

In the examples appearing in the printed manual, symbol statements specify COLOR=BLACK and order the plot symbols as follows: dot, square, triangle, circle, plus, x, diamond, and star.

Where to Turn for More Information

This section describes other sources of information about SAS/Genetics software.

Online Help System

You can access online help information about SAS/Genetics software in two ways. You can select **SAS System Help** from the **Help** pull-down menu and then select **SAS/Genetics Software** from the list of available topics. Or, you can bring up a command line and issue the command **help Genetics** to bring up an index to the statistical procedures, or issue the command **help ALLELE** (or another procedure name) to bring up the help for that particular procedure. Note that the online help includes syntax and some essential overview and detail material.

SAS Institute Technical Support Services

As with all SAS Institute products, the SAS Institute Technical Support staff is available to respond to problems and answer technical questions regarding the use of SAS/Genetics software.

Related SAS Software

Many features not found in SAS/Genetics software are available in other parts of the SAS System. If you do not find something you need in SAS/Genetics software, try looking for the feature in the following SAS software products.

Base SAS Software

The features provided by SAS/Genetics software are in addition to the features provided by Base SAS software. Many data management and reporting capabilities you will need are part of Base SAS software. Refer to *SAS Language Reference: Concepts*, *SAS Language Reference: Dictionary*, and the *SAS Procedures Guide* for documentation of Base SAS software.

SAS DATA Step

The DATA step is your primary tool for reading and processing data in the SAS System. The DATA step provides a powerful general-purpose programming language that enables you to perform all kinds of data processing tasks. The DATA step is documented in *SAS Language Reference: Concepts*.

Base SAS Procedures

Base SAS software includes many useful SAS procedures. Base SAS procedures are documented in the *SAS Procedures Guide*. The following is a list of Base SAS procedures you may find useful:

CHART	for printing charts and histograms
CONTENTS	for displaying the contents of SAS data sets
CORR	for computing correlations
FREQ	for computing frequency crosstabulations
MEANS	for computing descriptive statistics and summarizing or collapsing data over cross sections
PRINT	for printing SAS data sets
SORT	for sorting SAS data sets
TABULATE	for printing descriptive statistics in tabular format
TRANSPOSE	for transposing SAS data sets
UNIVARIATE	for computing descriptive statistics

SAS/GRAPH Software

SAS/GRAPH software includes procedures that create two- and three-dimensional high-resolution color graphics plots and charts. You can generate output that graphs the relationship of data values to one another, enhance existing graphs, or simply create graphics output that is not tied to data.

SAS/IML Software

SAS/IML software gives you access to a powerful and flexible programming language (Interactive Matrix Language) in a dynamic, interactive environment. The fundamental object of the language is a data matrix. You can use SAS/IML software interactively (at the statement level) to see results immediately, or you can store statements in a module and execute them later. The programming is dynamic because necessary activities such as memory allocation and dimensioning of matrices are done automatically. SAS/IML software is of interest to users of SAS/Genetics software because it enables you to program your own methods in the SAS System.

SAS/INSIGHT Software

SAS/INSIGHT software is a highly interactive tool for data analysis. You can explore data through a variety of interactive graphs including bar charts, scatter plots, box plots, and three-dimensional rotating plots. You can examine distributions and perform parametric and nonparametric regression, analyze general linear models and generalized linear models, examine correlation matrices, and perform principal component analyses. Any changes you make to your data show immediately in all graphs and analyses. You can also configure SAS/INSIGHT software to produce graphs and analyses tailored to the way you work.

SAS/INSIGHT software may be of interest to users of SAS/Genetics software for interactive graphical viewing of data, editing data, exploratory data analysis, and checking distributional assumptions.

SAS/STAT Software

SAS/STAT software includes procedures for a wide range of statistical methodologies including

- logistic and linear regression
- censored regression
- principal component analysis
- variance component analysis
- cluster analysis
- contingency table analysis
- categorical data analysis: log-linear and conditional logistic models
- general linear models
- linear and nonlinear mixed models
- generalized linear models
- multiple hypothesis testing

SAS/STAT software is of interest to users of SAS/Genetics software because many statistical methods for analyzing genetics data not included in SAS/Genetics software are provided in SAS/STAT software.

Chapter 2
The ALLELE Procedure

Chapter Contents

Chapter 2
The ALLELE Procedure

Overview

The ALLELE procedure performs preliminary analyses on genetic marker data. These analyses serve to characterize the markers themselves or the population from which they were sampled, and can also serve as the basis for joint analyses on markers and traits. A *genetic marker* is any heritable unit that obeys the laws of transmission genetics, and the analyses presented here assume the marker genotypes are determined without error. With an underlying assumption of random sampling, the analyses rest on the multinomial distribution of marker alleles, and many standard statistical techniques can be invoked with little modification. The ALLELE procedure uses the notation and concepts described by Weir (1996); this is the reference for all equations and methods not otherwise cited.

Data are usually collected at the genotypic level but interest is likely to be centered on the constituent alleles, so the first step is to construct tables of allele and genotype frequencies. When alleles are independent within individuals, that is when there is Hardy-Weinberg equilibrium (HWE), analyses can be conducted at the allelic level. For this reason the ALLELE procedure allows for Hardy-Weinberg testing, although testing is also recommended as a means for detecting possible errors in data.

PROC ALLELE calculates the PIC, heterozygosity, and allelic diversity measures that serve to give an indication of marker informativeness. Such measures can be useful in determining which markers to use for further linkage or association testing with a trait. High values of these measures are a sign of marker informativeness, which is a desirable property in linkage and association tests.

Associations between markers may also be of interest. PROC ALLELE provides tests and various statistics for the association, also called the linkage disequilibrium, between each pair of markers. These statistics can be formed either by using haplotypes that are given in the data, by estimating the haplotype frequencies, or by using only genotypic information.

Getting Started

Example

Suppose you have genotyped 25 individuals at five markers. You want to examine some basic properties of these markers, such as whether they are in HWE, how many alleles each has, what genotypes appear in the data, and if there is linkage disequilibrium between any pairs of markers. You have ten columns of data, with the first two columns containing the set of alleles at the first marker, the next two columns containing the set of alleles for the second marker, and so on. There is one row per each individual. You input your data as follows:

```
data markers;
   input (a1-a10) ($);
   datalines;
B  B  A  B  B  B  A  A  B  B
A  A  B  B  A  B  A  B  C  C
B  B  A  A  B  B  B  B  A  C
A  B  A  B  A  B  A  B  A  B
A  A  A  B  A  B  B  B  C  C
B  B  A  A  A  B  A  B  C  C
A  B  B  B  A  B  A  A  A  B
A  B  A  A  A  A  A  A  A  A
B  B  A  A  A  A  A  B  B  B
A  B  A  B  A  B  B  B  A  C
A  A  A  B  A  A  A  B  B  C
B  B  A  B  A  B  A  B  A  C
A  B  B  B  A  A  A  B  A  C
B  B  B  B  A  A  A  A  A  B
A  B  A  A  A  B  A  A  C  C
A  B  A  A  A  B  A  B  C  C
B  B  A  A  A  A  A  B  A  A
A  A  A  B  A  A  A  B  A  B
A  B  A  A  A  A  B  B  C  C
A  A  A  A  A  A  A  A  B  B
A  B  B  B  A  A  A  A  C  C
A  B  A  B  A  B  A  A  B  B
B  B  A  B  A  B  A  A  A  C
A  B  A  A  A  B  A  B  A  C
A  B  B  B  B  B  A  B  B  B
;
```

You can now use PROC ALLELE to examine the frequencies of alleles and genotypes in your data, and see if these frequencies are occurring in proportions you would expect. The following statements will perform the analysis you want:

```
proc allele data=markers outstat=ld prefix=Marker
            perms=10000 boot=1000 seed=123;
   var a1-a10;
run;

proc print data=ld;
run;
```

This analysis is using 10,000 permutations to approximate an exact p-value for the HWE test as well as 1,000 bootstrap samples to obtain the confidence interval for the allele frequencies and one-locus Hardy-Weinberg disequilibrium (HWD) coefficients. The starting seed for the random number generator is 123. The PREFIX= option requests that the five markers be named Marker1–Marker5. Since the BOOTSTRAP= option is specified but the ALPHA= option is omitted, a 95% confidence interval is calculated by default.

All five markers are included in the analysis since the ten variables containing the alleles for those five markers were specified in the VAR statement.

The marker data can alternatively be read in as columns of genotypes instead of columns of alleles using the GENOCOL and DELIMITER= options in the PROC ALLELE statement, with just one column per each marker. The following DATA step and SAS code could be used to produce the same output using data in this alternative format:

```
data markers;
   input (g1-g5) ($);
   datalines;
B/B  A/B  B/B  A/A  B/B
A/A  B/B  A/B  A/B  C/C
B/B  A/A  B/B  B/B  A/C
A/B  A/B  A/B  A/B  A/B
A/A  A/B  A/B  B/B  C/C
B/B  A/A  A/B  A/B  C/C
A/B  B/B  A/B  A/A  A/B
A/B  A/A  A/A  A/A  A/A
B/B  A/A  A/A  A/B  B/B
A/B  A/B  A/B  B/B  A/C
A/A  A/B  A/A  A/B  B/C
B/B  A/B  A/B  A/B  A/C
A/B  B/B  A/A  A/B  A/C
B/B  B/B  A/A  A/A  A/B
A/B  A/A  A/B  A/A  C/C
A/B  A/A  A/B  A/B  C/C
B/B  A/A  A/A  A/B  A/A
A/A  A/B  A/A  A/B  A/B
A/B  A/A  A/A  B/B  C/C
A/A  A/A  A/A  A/A  B/B
A/B  B/B  A/A  A/A  C/C
A/B  A/B  A/B  A/A  B/B
B/B  A/B  A/B  A/A  A/C
A/B  A/A  A/B  A/B  A/C
A/B  B/B  B/B  A/B  B/B
;


proc allele data=markers outstat=ld prefix=Marker
            perms=10000 boot=1000 seed=123
            genocol delimiter=’/’;
   var g1-g5;
run;

proc print data=ld;
run;
```

Note that the DELIMITER= option, which indicates the character or string that separates the alleles comprising a genotype, could have been omitted in this example since ’/’ is the default.

The results from the analysis are as follows.

```
                              The ALLELE Procedure

                                 Marker Summary

                                                   ----------Test for HWE---------
         Number  Number
             of      of         Hetero-   Allelic       Chi-             Pr >    Prob
Locus     Indiv Alleles     PIC zygosity Diversity     Square      DF   ChiSq   Exact

Marker1      25       2 0.3714   0.4800    0.4928     0.0169       1 0.8967 1.0000
Marker2      25       2 0.3685   0.3600    0.4872     1.7041       1 0.1918 0.2262
Marker3      25       2 0.3546   0.4800    0.4608     0.0434       1 0.8350 1.0000
Marker4      25       2 0.3648   0.4800    0.4800     0.0000       1 1.0000 1.0000
Marker5      25       3 0.5817   0.4400    0.6552     9.3537       3 0.0249 0.0106
```

Figure 2.1. Marker Summary for the ALLELE Procedure

Figure 2.1 displays information about the five markers. From this output, you can conclude that Marker5 is the only one showing significant departure from HWE.

```
                               Allele Frequencies

                                             Standard     95% Confidence
         Locus      Allele    Frequency        Error          Limits

         Marker1    A            0.4400       0.0711    0.3000    0.5800
         Marker1    B            0.5600       0.0711    0.4200    0.7000
         Marker2    A            0.5800       0.0784    0.4200    0.7400
         Marker2    B            0.4200       0.0784    0.2600    0.5800
         Marker3    A            0.6400       0.0665    0.5200    0.7600
         Marker3    B            0.3600       0.0665    0.2400    0.4800
         Marker4    A            0.6000       0.0693    0.4600    0.7400
         Marker4    B            0.4000       0.0693    0.2600    0.5400
         Marker5    A            0.2800       0.0637    0.1400    0.4200
         Marker5    B            0.3000       0.0800    0.1600    0.4600
         Marker5    C            0.4200       0.0833    0.2800    0.6000
```

Figure 2.2. Allele Frequencies for the ALLELE Procedure

Figure 2.2 displays the allele frequencies for each marker with their standard errors and the lower and upper limits of the 95% confidence interval.

```
                              Genotype Frequencies

                                             HWD    Standard      95% Confidence
Locus      Genotype    Frequency           Coeff       Error          Limits

Marker1    A/A            0.2000          0.0064      0.0493     -0.0916      0.0956
Marker1    A/B            0.4800          0.0064      0.0493     -0.0916      0.0956
Marker1    B/B            0.3200          0.0064      0.0493     -0.0916      0.0956
Marker2    A/A            0.4000          0.0636      0.0477     -0.0336      0.1484
Marker2    A/B            0.3600          0.0636      0.0477     -0.0336      0.1484
Marker2    B/B            0.2400          0.0636      0.0477     -0.0336      0.1484
Marker3    A/A            0.4000         -0.0096      0.0457     -0.1044      0.0800
Marker3    A/B            0.4800         -0.0096      0.0457     -0.1044      0.0800
Marker3    B/B            0.1200         -0.0096      0.0457     -0.1044      0.0800
Marker4    A/A            0.3600          0.0000      0.0480     -0.0916      0.0864
Marker4    A/B            0.4800          0.0000      0.0480     -0.0916      0.0864
Marker4    B/B            0.1600          0.0000      0.0480     -0.0916      0.0864
Marker5    A/A            0.0800          0.0016      0.0405     -0.0756      0.0816
Marker5    A/B            0.1600          0.0040      0.0337     -0.0664      0.0636
Marker5    A/C            0.2400         -0.0024      0.0380     -0.0736      0.0680
Marker5    B/B            0.2000          0.1100      0.0445      0.0144      0.1884
Marker5    B/C            0.0400          0.1060      0.0282      0.0440      0.1564
Marker5    C/C            0.2800          0.1036      0.0453      0.0096      0.1884
```

Figure 2.3. Genotype Frequencies for the ALLELE Procedure

Figure 2.3 displays the genotype frequencies for each marker with the associated disequilibrium coefficient, its standard error, and the 95% confidence limits.

```
Obs    Locus1     Locus2     NIndiv    Test     ChiSq     DF    ProbChi    ProbEx

  1    Marker1    Marker1      25      HWE     0.01687     1    0.89667    1.0000
  2    Marker1    Marker2      25      LD      1.05799     1    0.30367    0.6707
  3    Marker1    Marker3      25      LD      1.42074     1    0.23328    0.6524
  4    Marker1    Marker4      25      LD      0.33144     1    0.56481    0.9668
  5    Marker1    Marker5      25      LD      2.29785     2    0.31698    0.8398
  6    Marker2    Marker2      25      HWE     1.70412     1    0.19175    0.2262
  7    Marker2    Marker3      25      LD      0.13798     1    0.71030    0.7242
  8    Marker2    Marker4      25      LD      1.34100     1    0.24686    0.9015
  9    Marker2    Marker5      25      LD      1.13574     2    0.56673    0.5503
 10    Marker3    Marker3      25      HWE     0.04340     1    0.83497    1.0000
 11    Marker3    Marker4      25      LD      0.46296     1    0.49624    0.9323
 12    Marker3    Marker5      25      LD      0.95899     2    0.61909    0.2624
 13    Marker4    Marker4      25      HWE     0.00000     1    1.00000    1.0000
 14    Marker4    Marker5      25      LD      6.16071     2    0.04594    0.9235
 15    Marker5    Marker5      25      HWE     9.35374     3    0.02494    0.0106
```

Figure 2.4. Testing for Disequilibrium Using the ALLELE Procedure

Figure 2.4 displays the output data set created using the OUTSTAT= option of the PROC ALLELE statement. This data set contains the statistics for testing individual markers for HWE and marker pairs for linkage disequilibrium.

Syntax

The following statements are available in PROC ALLELE.

> **PROC ALLELE** < *options* > **;**
>   **BY** *variables* **;**
>   **VAR** *variables* **;**

Items within angle brackets (< >) are optional, and statements following the PROC ALLELE statement can appear in any order. The VAR statement is required. The syntax of each statement is described in the following section in alphabetical order after the description of the PROC ALLELE statement.

PROC ALLELE Statement

PROC ALLELE < *options* > **;**

You can specify the following options in the PROC ALLELE statement.

ALLELEMIN=*number*
AMIN=*number*

indicates that only alleles with a frequency greater than or equal to *number* should be included in the "Allele Frequencies" table. By default, any allele that appears in a nonmissing genotype in the sample is included in the table. The value of *number* must be between 0 and 1.

ALPHA=*number*

specifies that a confidence level of $100(1-\mathit{number})\%$ is to be used in forming bootstrap confidence intervals for estimates of allele frequencies and disequilibrium coefficients. The value of *number* must be between 0 and 1, and is set to 0.05 by default.

BOOTSTRAP=*number*
BOOT=*number*

indicates that bootstrap confidence intervals should be formed for the estimates of allele frequencies and one-locus disequilibrium coefficients using *number* random samples. One thousand samples are usually recommended to form confidence intervals. If this statement is omitted, no confidence limits are reported.

CORRCOEFF

requests that the "Linkage Disequilibrium Measures" table be displayed and contain the correlation coefficient r, a linkage disequilibrium measure.

DATA=*SAS-data-set*

names the input SAS data set to be used by PROC ALLELE. The default is to use the most recently created data set.

DELIMITER=*’string’*

indicates the string that is used to separate the two alleles that comprise the genotypes contained in the variables specified in the VAR statement. This option is ignored if GENOCOL is not specified.

DELTA

requests that the “Linkage Disequilibrium Measures” table be displayed and contain the population attributable risk δ, a linkage disequilibrium measure. This option is ignored if HAPLO=NONE.

DPRIME

requests that the “Linkage Disequilibrium Measures” table be displayed and contain Lewontin’s D', a linkage disequilibrium measure.

GENOCOL

indicates that columns specified in the VAR statement contain genotypes instead of alleles. When this option is specified, there is one column per marker. The genotypes must consist of the two alleles separated by a delimiter.

GENOMIN=*number*
GMIN=*number*

indicates that only genotypes with a frequency greater than or equal to *number* should be included in the “Genotype Frequencies” table. By default, any genotype that appears at least once in the sample is included in the table. The value of *number* must be between 0 and 1.

HAPLO=NONE
HAPLO=EST
HAPLO=GIVEN

indicates whether haplotypes frequencies should not be used, haplotype frequencies should be estimated, or observed haplotype frequencies in the data should be used. This option affects all linkage disequilibrium tests and measures. By default or when HAPLO=NONE is specified, the composite linkage disequilibrium (CLD) coefficient is used in place of the usual linkage disequilibrium (LD) coefficient. In addition, the composite haplotype frequencies are used to form the linkage disequilibrium measures indicated by the options CORRCOEFF and DPRIME. When HAPLO=EST, the maximum likelihood estimates of the haplotype frequencies are used to calculate the LD test statistic as well as the LD measures. The HAPLO=GIVEN option indicates that the haplotypes have been observed, and thus the observed haplotype frequencies are used in the LD test statistic and measures.

When HAPLO=GIVEN, haplotypes are denoted in the data in the following manner according to the type of input data used:

- If you omit the experimental TALL option in the PROC ALLELE statement, then the alleles that comprise all alleles comprising one of an individual’s two haplotypes must all be in the first of the two variables listed for each marker, and alleles of the other haplotype in the second of the two variables listed for each marker. Similarly, if the GENOCOL option is used, the alleles comprising one haplotype should all be the first allele listed in each genotype, and alleles of the other haplotype listed second.

- If you specify the TALL option, then the alleles that comprise one haplotype for an individual must all be in the first variable in the VAR statement and all the alleles in the other haplotype must be in the second variable in the VAR statement. When the GENOCOL option is also specified, the alleles comprising one haplotype should all be the first allele listed in the genotype, and alleles of the other haplotype listed second.

HAPLOMIN=*number*
HMIN=*number*

indicates that only haplotypes with a frequency greater than or equal to *number* should be included in the "Linkage Disequilibrium Measures" table. By default, any haplotype that appears in the sample (or is estimated to appear at least once) is included in the table. The value of *number* must be between 0 and 1.

Experimental

INDIVIDUAL=*variable*
INDIV=*variable*

specifies the individual ID variable when using the experimental TALL option. This variable may be character or numeric.

Experimental

MARKER=*variable*

specifies the marker ID variable when using the experimental TALL option. This variable contains the names of the markers that are used in all output and may be character or numeric.

MAXDIST=*number*

specifies the maximum number of markers apart that a pair of markers can be in order to perform any linkage disequilibrium calculations. For example, if MAXDIST=1 is specified, linkage disequilibrium measures and statistics are calculated only for pairs of markers that are one apart, such as M1 and M2, M2 and M3, and so on. The number specified must be an integer and is set to 50 markers by default. This option assumes that markers are specified in the VAR statement in the physical order in which they appear on a chromosome or across the genome.

NDATA=*SAS-data-set*

names the input SAS data set containing names, or identifiers, for the markers used in the output. There must be a NAME variable in this data set, which should contain the same number of rows as there are markers in the input data set specified in the DATA= option. When there are fewer rows than there are markers, markers without a name are named using the PREFIX= option. Likewise, if there is no NDATA= data set specified, the PREFIX= option is used. Note that this data set is ignored if the experimental TALL option is specified in the PROC ALLELE statement. In that case, the marker variable names are taken from the marker ID variable specified in the MARKER= option.

NOFREQ

suppresses the display of the "Allele Frequencies" and the "Genotype Frequencies" tables. See the section "Displayed Output" on page 35 for a detailed description of these tables.

NOPRINT

suppresses the normal display of results. Note that this option temporarily disables the Output Delivery System (ODS).

OUTSTAT=*SAS-data-set*

names the output SAS data set containing the disequilibrium statistics, for both within-marker and between-marker disequilibria.

PERMS=*number*
EXACT=*number*

indicates that Monte Carlo estimates of the exact p-values for the disequilibrium tests should be calculated using *number* permutations. Large values of *number* (10,000 or more) are usually recommended for accuracy, but long execution times may result, particularly with large data sets. When this option is omitted, no permutations are performed and asymptotic p-values are reported. If HAPLO=EST, then only the exact tests for Hardy-Weinberg equilibrium are performed; the exact tests for linkage disequilibrium cannot be performed since haplotypes are unknown.

PREFIX=*prefix*

specifies a prefix to use in constructing names for marker variables in all output. For example, if PREFIX=VAR, the names of the variables are VAR1, VAR2, ..., VAR*n*. Note that this option is ignored when the NDATA= option is specified, unless there are fewer names in the NDATA data set than there are markers; it is also ignored if the experimental TALL option is specified, in which case the marker variable names are taken from the marker ID variable specified in the MARKER= option. Otherwise, if this option is omitted, PREFIX=M is the default when variables contain alleles; if GENOCOL is specified, then the names of the variables specified in the VAR statement are used as the marker names.

PROPDIFF

requests that the "Linkage Disequilibrium Measures" table be displayed and contain the proportional difference d, a linkage disequilibrium measure. This option is ignored if HAPLO=NONE.

SEED=*number*

specifies the initial seed for the random number generator used for permuting the data in the exact tests and for the bootstrap samples. The value for *number* must be an integer; the computer clock time is used if the option is omitted or the integer specified is less than or equal to 0. For more details about seed values, refer to *SAS Language Reference: Concepts*.

TALL *Experimental*

indicates that the input data set is of an alternative format. This format contains the following columns: two containing marker alleles (or one containing marker genotypes if GENOCOL is specified), one for the marker identifier, and one for the individual identifier. The experimental MARKER= and INDIV= options must also be specified for this option to be in effect. Note that when this option is used, the DATA= data set must first be sorted by any BY variables, then sorted by the marker ID variable, then the individual ID variable.

YULESQ

requests that the "Linkage Disequilibrium Measures" table be displayed and contain Yule's Q, a linkage disequilibrium measure. This option is ignored if HAPLO=NONE.

BY Statement

BY *variables* **;**

You can specify a BY statement with PROC ALLELE to obtain separate analyses on observations in groups defined by the BY variables. When a BY statement appears, the procedure expects the input data set to be sorted in the order of the BY variables. The *variables* are one or more variables in the input data set.

If your input data set is not sorted in ascending order, use one of the following alternatives:

- Sort the data using the SORT procedure with a similar BY statement.
- Specify the BY statement option NOTSORTED or DESCENDING in the BY statement for the ALLELE procedure. The NOTSORTED option does not mean that the data are unsorted but rather that the data are arranged in groups (according to values of the BY variables) and that these groups are not necessarily in alphabetical or increasing numeric order.
- Create an index on the BY variables using the DATASETS procedure (in base SAS software).

For more information on the BY statement, refer to the discussion in *SAS Language Reference: Concepts*. For more information on the DATASETS procedure, refer to the discussion in the *SAS Procedures Guide*.

VAR Statement

VAR *variables* **;**

The VAR statement identifies the variables containing either the marker alleles, or the marker genotypes if GENOCOL is specified. The following number of variables should be specified in this statement for a data set containing m markers according to whether the options GENOCOL and TALL are used:

- When both GENOCOL and TALL are specified, there should be one variable named containing marker genotypes.
- When only TALL is specified, there should be two variables named containing marker alleles.
- When only GENOCOL is specified, there should be m variables named, one for each marker containing marker genotypes.

- When neither option is specified, there should be $2m$ variables named, two for each marker containing marker alleles.

All variables specified must be of the same type, either character or numeric.

Details

Statistical Computations

Frequency Estimates

A marker locus **M** may have a series of alleles M_u, $u = 1, ..., k$. A sample of n individuals may therefore have several different genotypes at the locus, with n_{uv} copies of type M_u/M_v. The number n_u of copies of allele M_u can be found directly by summation: $n_u = 2n_{uu} + \sum_{v \neq u} n_{uv}$. The sample frequencies are written as $\tilde{p}_u = n_u/(2n)$ and $\tilde{P}_{uv} = n_{uv}/n$. The $\tilde{P}_{uv}$'s are unbiased maximum likelihood estimates (MLEs) of the population proportions P_{uv}.

The variance of the sample allele frequency $\tilde{p}_u$ is calculated as

$$\text{Var}(\tilde{p}_u) = \frac{1}{2n}(p_u + P_{uu} - 2p_u^2)$$

and can be estimated by replacing p_u and P_{uu} with their sample values $\tilde{p}_u$ and $\tilde{P}_{uu}$. The variance of the sample genotype frequency $\tilde{P}_{uv}$ is not generally calculated; instead, an MLE of the HWD coefficient D_{uv} for alleles M_u and M_v is calculated as

$$\hat{D}_{uv} = \begin{cases} \tilde{P}_{uv} - \tilde{p}_u\tilde{p}_v, & u = v \\ \tilde{p}_u\tilde{p}_v - \frac{1}{2}\tilde{P}_{uv}, & u \neq v \end{cases}$$

and the MLE's variance is estimated using one of the following formulas, depending on whether the two alleles are the same or different:

$$\begin{aligned} \text{Var}(\hat{D}_{uu}) &= \frac{1}{n}\Big[\tilde{p}_u^2(1-\tilde{p}_u)^2 + (1-2\tilde{p}_u)^2\hat{D}_{uu} - \hat{D}_{uu}^2\Big] \\ \text{Var}(\hat{D}_{uv}) &= \frac{1}{2n}\Big\{\tilde{p}_u\tilde{p}_v(1-\tilde{p}_u)(1-\tilde{p}_v) + \sum_{w \neq u,v}(\tilde{p}_u^2\hat{D}_{vw} + \tilde{p}_v^2\hat{D}_{uw}) \\ &\quad - \left[(1-\tilde{p}_u-\tilde{p}_v)^2 - 2(\tilde{p}_u-\tilde{p}_v)^2\right]\hat{D}_{uv} + \tilde{p}_u^2\tilde{p}_v^2 - 2\hat{D}_{uv}^2\Big\} \end{aligned}$$

The standard error, the square root of the variance, is reported for the sample allele frequencies and the disequilibrium coefficient estimates. When the BOOTSTRAP= option of the PROC ALLELE statement is specified, bootstrap confidence intervals are formed by resampling individuals from the data set and are reported for these estimates, with the $100(1-\alpha)\%$ confidence level given by the ALPHA=α option (or $\alpha = 0.05$ by default).

Measures of Marker Informativeness

Polymorphism Information Content

The polymorphism information content (PIC) measures the probability of differentiating the allele transmitted by a given parent to its child given the marker genotype of father, mother, and child (Botstein et al. 1980). It is computed as

$$\text{PIC} = 1 - \sum_{u=1}^{k} \tilde{p}_u^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{p}_u^2 \tilde{p}_v^2$$

Heterozygosity

The heterozygosity, sometimes called the observed heterozygosity, is simply the proportion of heterozygous individuals in the data set and is calculated as

$$\text{Het} = 1 - \sum_{u=1}^{k} \tilde{P}_{uu}$$

Allelic Diversity

The allelic diversity, sometimes called the expected heterozygosity, is the expected proportion of heterozygous individuals in the data set when HWE holds and is calculated as

$$\text{Div} = 1 - \sum_{u=1}^{k} \tilde{p}_u^2$$

Testing for Hardy-Weinberg Equilibrium

Under ideal population conditions, the two alleles an individual receives, one from each parent, are independent so that $P_{uu} = p_u^2$ and $P_{uv} = 2p_u p_v, u \neq v$. The factor of 2 for heterozygotes recognizes the fact that M_u/M_v and M_v/M_u genotypes are generally indistinguishable. This statement about allelic independence within loci is called Hardy-Weinberg equilibrium (HWE). Forces such as selection, mutation, and migration in a population or nonrandom mating can cause departures from HWE. Two methods are used here for testing a marker for HWE, both of which can accommodate any number of alleles. Both methods are testing the hypothesis that $P_{uu} = p_u^2$ and $P_{uv} = 2p_u p_v, u \neq v$ for all $u, v = 1, ..., k$.

Chi-Square Goodness-of-Fit Test

The chi-square goodness-of-fit test can be used to test markers for HWE. The chi-square statistic

$$X_T^2 = \sum_u \frac{(n_{uu} - n\tilde{p}_u^2)^2}{n\tilde{p}_u^2} + \sum_u \sum_{v>u} \frac{(n_{uv} - 2n\tilde{p}_u\tilde{p}_v)^2}{2n\tilde{p}_u\tilde{p}_v}$$

has $k(k-1)/2$ degrees of freedom where k is the number of alleles at the marker locus.

Permutation Version of Exact Test

The permutation version of the exact test given by Guo and Thompson (1992) is based on the conditional probability of genotype counts given allelic counts and the hypothesis of allelic independence. The test statistic is

$$T = \frac{n!}{(2n)!} \frac{2^h \prod_u n_u!}{\prod_{u,v} n_{uv}!}$$

where $h = \sum_u \sum_{v \neq u} n_{uv}$ is the number of heterozygous individuals. Significance levels are calculated by the Monte Carlo permutation procedure. The $2n$ alleles are randomly permuted the number of times indicated in the PERMS= option to form new sets of n genotypes. The significance level is then calculated as the proportion of times the value of T for each set of permuted data exceeds the value of T for the actual data. You can indicate the random seed used to randomly permute the data in the SEED= option of the PROC ALLELE statement.

Linkage Disequilibrium (LD)

The set of genetic material an individual receives from each parent contains an allele at every locus, and statements can be made about these allelic combinations, or haplotypes. The probability p_{uv} (called the gametic or haplotype frequency) that an individual receives the haplotype $M_u N_v$ for marker loci **M** and **N** can be compared to the product of the probabilities that each allele is received. The difference is the linkage, or gametic, disequilibrium (LD) coefficient D_{uv} for those two alleles: $D_{uv} = p_{uv} - p_u p_v$. There is a general expectation that the amount of linkage disequilibrium is inversely related to the distance between the two loci, but there are many other factors that may affect disequilibrium. There may even be disequilibrium between alleles at loci that are located on different chromosomes. Note that these tests and measures will be calculated only for pairs of markers at most d markers apart, where d is the integer specified in the MAXDIST= option of the PROC ALLELE statement, or 50 by default.

Table 2.1 displays how the HAPLO= option of the PROC ALLELE statement interacts with the linkage disequilibrium calculations. These calculations are discussed in more detail in the following two sections.

Table 2.1. Interaction of HAPLO= option with LD calculations

HAPLO= Option	LD Test Statistic	LD Exact Test	Estimate of Haplotype Freq
GIVEN	$\tilde{D}_{uv}$	Permutes alleles to form new 2-locus haplotypes	Observed freq, $\tilde{p}_{uv}$
EST	$\hat{D}_{uv}$	Not performed	Estimated freq, $\hat{p}_{uv}$
NONE	$\tilde{\Delta}_{uv}$	Permutes genotypes to form new 2-locus genotypes	Composite freq, $\tilde{p}^*_{uv}$

Tests

When haplotypes are known, the HAPLO=GIVEN option should be included in the PROC ALLELE statement so that the linkage disequilibrium can be computed directly by substituting the observed frequencies $\tilde{p}_{uv}$, $\tilde{p}_u$, and $\tilde{p}_v$ into the equation in the preceding section for D_{uv}. This creates the MLE, $\tilde{D}_{uv}$, of the LD coefficient between a pair of alleles at different markers. PROC ALLELE calculates an overall chi-square statistic to test that all of the D_{uv}'s between two markers are zero as follows:

$$X_T^2 = \sum_{u=1}^{k} \sum_{v=1}^{l} \frac{(2n)\tilde{D}_{uv}^2}{\tilde{p}_u \tilde{p}_v}$$

which has $(k-1)(l-1)$ degrees of freedom for markers with k and l alleles, respectively.

There is also a Monte Carlo estimate of the exact test available when haplotypes are known. An estimate of the exact p-value for testing the hypothesis in the preceding paragraph can be calculated by conditioning on the allele counts as with the permutation version of the exact test for HWE. The conditional probability of the haplotype counts is then

$$T = \frac{\prod_u n_u! \prod_v n_v!}{(2n)! \prod_{u,v} n_{uv}!}$$

and the significance level is obtained again by permuting the alleles at one locus to form $2n$ new two-locus haplotypes. You can indicate the number of permutations that are used in the PERMS= option of the PROC ALLELE statement and the random seed used to randomly permute the data in the SEED= option of the PROC ALLELE statement.

When it is requested that haplotype frequencies be estimated with the HAPLO=EST option, D_{uv} is estimated using $\hat{D}_{uv} = \hat{p}_{uv} - \tilde{p}_u \tilde{p}_v$, where $\hat{p}_{uv}$ is the MLE of p_{uv} assuming HWE. The estimate $\hat{p}_{uv}$ is calculated according to the method described by Weir and Cockerham (1979). Again, a chi-square test statistic can be calculated to test that all of the D_{uv}'s between a pair of markers are zero as

$$X_T^2 = \sum_{u=1}^{k} \sum_{v=1}^{l} \frac{n\hat{D}_{uv}^2}{\tilde{p}_u \tilde{p}_v}$$

which has $(k-1)(l-1)$ degrees of freedom for markers with k and l alleles, respectively. No exact test is available when haplotype frequencies are estimated.

The HAPLO=NONE option indicates that haplotypes are unknown and $\hat{D}_{uv}$ should not be used in the tests for LD between pairs of markers. Instead of using the estimated haplotype frequencies which assumes HWE, a test can be formed using the composite linkage disequilibrium (CLD) coefficient Δ_{uv} that does not require this assumption and uses only allele and two-locus genotype frequencies. The MLE $\tilde{\Delta}_{uv}$

of Δ_{uv} can be calculated as described by Weir (1979), and a chi-square statistic that tests all Δ_{uv}'s between a pair of markers are zero can be formed as follows:

$$X_T^2 = \sum_{u=1}^{k} \sum_{v=1}^{l} \frac{n\tilde{\Delta}_{uv}^2}{\tilde{p}_u \tilde{p}_v}$$

which has $(k-1)(l-1)$ degrees of freedom for markers with k and l alleles, respectively.

A permutation version of the exact test for CLD is also available, where the conditional probability of the two-locus genotypes given the one-locus genotypes is

$$T = \frac{\prod_{r,s} n_{rs}! \prod_{u,v} n_{uv}!}{n! \prod_{r,s,u,v} n_{rsuv}!}$$

where n_{rsuv} is the count of $M_r M_s N_u N_v$ genotypes, n_{rs} is the count of M_r/M_s genotypes, and n_{uv} is the count of N_u/N_v genotypes. An estimate of the exact significance level is obtained by permuting the genotypes at one of the loci to create a distribution of the T's (Zaykin, Zhivotovsky, and Weir 1995). Note that this procedure is also testing for nonzero trigenic and quadrigenic disequilibrium terms, so significance may not necessarily imply the presence of CLD.

Measures

PROC ALLELE offers five linkage disequilibrium measures to be calculated for each pair of alleles M_u and N_v located at loci **M** and **N** respectively: the correlation coefficient r, the population attributable risk δ, Lewontin's D', the proportional difference d, and Yule's Q. The five measures are discussed in Devlin and Risch (1995). Since these measures are designed for biallelic markers, the measures are calculated for each allele at locus **M** with each allele at locus **N**, where all other alleles at each loci are combined to represent one allele. Thus for each allele M_u in turn, $\tilde{p}_1$ is used as the frequency of allele M_u, and $\tilde{p}_2$ represents the frequency of "not M_u"; similarly for each N_v in turn, $\tilde{q}_1$ represents the frequency of allele N_v, and $\tilde{q}_2$ the frequency of "not N_v." All measures have the same numerator, $D = p_{11}p_{22} - p_{12}p_{21}$, the LD coefficient, which can be directly estimated using the observed haplotype frequencies $\tilde{p}_{uv}$ when HAPLO=GIVEN, or estimated using the MLEs of the haplotype frequencies $\hat{p}_{uv}$ assuming HWE when HAPLO=EST. The computations for the measures are as follows:

$$\begin{aligned}
r &= \frac{D}{(p_1 p_2 q_1 q_2)^{1/2}} \\
\delta &= \frac{D}{q_1 p_{22}} \\
D' &= \frac{D}{D_{\max}}, D_{\max} = \begin{cases} \min(p_1 q_2, q_1 p_2), & D > 0 \\ \min(p_1 q_1, q_2 p_2), & D < 0 \end{cases} \\
d &= \frac{D}{q_1 q_2} \\
Q &= \frac{D}{p_{11}p_{22} + p_{12}p_{21}}
\end{aligned}$$

with estimates of measures calculated by replacing parameters with their appropriate estimates. Under the default option HAPLO=NONE, the numerator D can be replaced by the CLD coefficient Δ, described in the preceding section, for measures r and D'. This statistic has bounds twice as large as D so the denominator for D' must be multiplied by a factor of 2. However, δ, d, and Q cannot be calculated when HAPLO=NONE.

Missing Values

An individual's genotype for a marker is considered missing if at least one of the alleles at the marker is missing. Any missing genotypes are excluded from all calculations, including the linkage disequilibrium statistics for all pairs that include the marker. However, the individual's nonmissing genotypes at other markers can be used as part of the calculations.

If the BOOTSTRAP= option is specified, any individuals with missing genotypes for all markers are excluded from resampling. All other individuals are included, which could result in different numbers of individuals with nonmissing genotypes for the same marker across different samples.

OUTSTAT= Data Set

The OUTSTAT= data set contains the following variables:

- the BY variables, if any
- Locus1 and Locus2, which contain the pair of markers for which the disequilibrium statistics are calculated
- NIndiv, which contains the number of individuals that have been genotyped at both the markers listed in Locus1 and Locus2 (that is, the number of individuals that have no missing alleles for the two loci)
- Test, which indicates which disequilibrium test is performed, HWE for individual markers (when Locus1 and Locus2 contain the same value) or LD for marker pairs
- ChiSq, which contains the chi-square statistic for testing for disequilibrium. If Locus1 and Locus2 contain the same marker, the test is for HWE within that locus. Otherwise, it is a test for linkage disequilibrium between the two loci.
- DF, which contains the degrees of freedom for the chi-square test
- ProbChi, which contains the p-value for the chi-square test
- ProbEx, which contains an estimate of the exact p-value for testing the pair of markers in Locus1 and Locus2 for disequilibrium. This variable is included in the OUTSTAT= data set only when the PERMS= parameter in the PROC ALLELE statement is a positive integer and HAPLO=NONE or HAPLO=GIVEN.

Displayed Output

This section describes the displayed output from PROC ALLELE. See the section "ODS Table Names" on page 36 for details about how this output interfaces with the Output Delivery System.

Marker Summary

The "Marker Summary" table lists information on each of the markers, including

- NIndiv, the number of individuals genotyped at the marker
- NAllele, the number of alleles at the marker
- PIC, the polymorphism information content (PIC) measure
- Het, the heterozygosity measure
- Div, the allelic diversity measure

as well as the following columns for the test for HWE:

- ChiSq, the chi-square statistic
- DF, the degrees of freedom for the chi-square test
- ProbChiSq, the p-value for the chi-square test
- ProbExact, an estimate of the exact p-value for the HWE test (only if the PERMS= option is specified in the PROC ALLELE statement)

Allele Frequencies

The "Allele Frequencies" table lists all the observed alleles for each marker, with an estimate of the allele frequency, the standard error of the frequency, and when the BOOTSTRAP= option is specified, the bootstrap lower and upper limits of the confidence interval for the frequency based on the confidence level determined by the ALPHA= option of the PROC ALLELE statement (0.95 by default).

Genotype Frequencies

The "Genotype Frequencies" table lists all the observed genotypes (denoted by the two alleles separated by a "/") for each marker, with the observed genotype frequency, an estimate of the disequilibrium coefficient D, the standard error of the estimate, and when the BOOTSTRAP= option is specified, the lower and upper limits of the bootstrap confidence interval for D based on the confidence level determined by the ALPHA= option of the PROC ALLELE statement (0.95 by default).

Linkage Disequilibrium Measures

The “Linkage Disequilibrium Measures” table lists the frequency of each haplotype at each marker pair (observed frequency when HAPLO=GIVEN and estimated frequency otherwise), an estimate of the LD coefficient D_{uv}, and whichever linkage disequilibrium measures are included in the PROC ALLELE statement (CORRCOEFF, DELTA, DPRIME, PROPDIFF, and YULESQ). Haplotypes are represented by the allele at the marker locus listed in Locus1 and the allele at the marker locus listed in Locus2 separated by a “-.” Note that this table can be quite large when there are many markers or markers with many alleles. For a data set with m markers, each having k_i alleles, $i = 1, ..., m$, the number of rows in the table is $\sum_{i=1}^{m-1} \sum_{j=i+1}^{m} k_i k_j$. The MAXDIST= option of the PROC ALLELE statement can be used to keep this table to a manageable size.

ODS Table Names

PROC ALLELE assigns a name to each table it creates, and you must use this name to reference the table when using the Output Delivery System (ODS). These names are listed in the following table.

Table 2.2. ODS Tables Created by the ALLELE Procedure

ODS Table Name	Description	PROC ALLELE option
MarkerSumm	Marker summary	default
AlleleFreq	Allele frequencies	default
GenotypeFreq	Genotype frequencies	default
LDMeasures	Linkage disequilibrium measures	CORRCOEFF, DELTA, DPRIME, PROPDIFF, or YULESQ

Examples

Example 2.1. Using the NDATA= Option with Microsatellites

The following is a subset of data from GAW12 (Wijsman et al. 2001) and contains 17 individuals’ genotypes at 14 microsatellite markers.

```
data gaw;
   input id m1-m14 / m15-m28;
   datalines;
 1 11 14  6  8  2  5  9  4  6  1  9  9  9  7
    3  5 10  1  4  6  5  9  1  1  3  5  6  2
 2  2 12  1  4  6  6  3  3  2  1 11 11  4 11
    2  2 13 11  2  1  9  9  1  5  6  1  2  5
 3  2 10  4  8  4  9  2  7  7  1  9  2  7 10
    2  2  7  7  6  8  9  4  5  1  7  2  6  2
 4  5 14  7  3  9 13  4  2  2  4 11  5  4  7
    4  5  7  6  8  2  9  9  1  6  4  1  8  9
 5 12 12  3  8  6  2  1  7  3  5  6 11  6  9
    5  2 13 16  7  1  9  4  1  1  7  1  1  2
 6  4  7  7  8  7 12  4  2  6  5  5 11  5 11
    2  4 15 11  1  1  9  2  6  5  7  6  1  5
 7  2 10  6  8  7  1  2  3  6  2  5  8  5  6
    5  6 13 10  1  8  9  3  1  6  7  7  2  6
 8  2 11  6  2  7  1  2  3  6  6 10 11 11  6
    4  2 11 11  4  5 11  2  3  2  1  4  1  2
 9  2  7  1  1  3  1  5  7  2  5  5 11 11 11
    2  6 11  2  1  6  4  9  5  5  4  2  5  9
10 11 12  2  4 13  3  1  2  4  9  5 10  7  5
    4  4  1  6  8  1  6 10  1  1  2  5  1  1
11 11  2  7  8  1  5  4  6  4  7  5 11 11  6
    5  4 16 13  7  4  5  6  6  1  1  4  1  1
12  2 12  6  8  2  7  3  2  7  5  2  8  9  6
    2  4  7 16  7  1 10  9  5  1  1  4  9  1
13 13 14  8  3 12 13  7  4  3  2  6 10  9  5
    4  4  2 14  8  8  3  6  5  1  1  6  6  2
14  7 10  6  5 10 13  8  3  5  5  9  9 11  6
    5  4 13 14  1  1  6  9  2  1  5  3  1  2
15 10 11  4  3  9  7  6  3  4  6 10  1  7  9
    2  2  2 14  6  1  9  2  1  1  6  7  5  2
16  2  5  2  7  7  2  2  9  2  2  2  6  9  5
    2  2  7  1  1  2  6  2  1  1  1  1  9  6
17 11  4  4  4  9  1  7  8  5  3  5  1 11  5
    6  5  2 12  1  5  9  9  1  5  7  7  6  1
;
```

Note that you can input the same data directly using the statement:

```
infile 'Genmrk22.1' delimiter="/ ";
```

in place of the DATALINES statement.

The actual names of the markers can be used, by creating a data set with the variable NAME containing these names.

```
data map;
   input name $ location;
   datalines;
D22G001   0.50
D22G002   0.79
D22G003   0.88
D22G004   1.02
D22G005   1.24
D22G006   2.20
D22G007   4.27
D22G008   5.85
D22G009   6.70
D22G010   9.36
D22G011  10.87
D22G012  11.67
D22G013  12.66
D22G014  15.89
;
```

Now an analysis using PROC ALLELE can be performed as follows:

```
proc allele data=gaw ndata=map nofreq perms=10000 seed=456;
   var m1-m28;
run;
```

This analysis produces summary statistics of the 14 markers and is using 10,000 permutations to approximate an exact p-value for the HWE test. The allele and genotype frequency output tables are suppressed with the NOFREQ option.

The results from the analysis are as follows. Note the names of the markers that are used.

Output 2.1.1. Summary of Microsatellites for the ALLELE Procedure

```
                              The ALLELE Procedure

                                 Marker Summary

                  Number     Number
                      of         of                 Hetero-       Allelic
        Locus      Indiv    Alleles        PIC     zygosity     Diversity

        D22G001       17          9     0.8384       0.9412        0.8547
        D22G002       17          8     0.8296       0.8824        0.8478
        D22G003       17         11     0.8749       0.9412        0.8858
        D22G004       17          9     0.8259       0.9412        0.8443
        D22G005       17          8     0.8272       0.8235        0.8460
        D22G006       17          8     0.8257       0.8235        0.8443
        D22G007       17          7     0.8012       0.9412        0.8253
        D22G008       17          5     0.6665       0.6471        0.7163
        D22G009       17         11     0.8788       0.8824        0.8893
        D22G010       17          7     0.7572       0.8235        0.7820
        D22G011       17          8     0.7274       0.8235        0.7509
        D22G012       17          5     0.5661       0.6471        0.6142
        D22G013       17          7     0.7965       0.8235        0.8201
        D22G014       17          6     0.7507       0.8824        0.7837

                                 Marker Summary

                       --------------Test for HWE--------------

                              Chi-                   Pr >       Prob
              Locus         Square          DF      ChiSq      Exact

              D22G001      32.5172          36     0.6350     0.8581
              D22G002      28.5222          28     0.4370     0.3868
              D22G003      48.2139          55     0.7295     0.7050
              D22G004      24.9692          36     0.9166     0.8361
              D22G005      20.9416          28     0.8278     0.9413
              D22G006      32.0018          28     0.2744     0.1102
              D22G007      19.7625          21     0.5363     0.5745
              D22G008      11.4619          10     0.3227     0.2525
              D22G009      52.1333          55     0.5849     0.3866
              D22G010      14.7227          21     0.8366     0.8624
              D22G011      19.0400          28     0.8969     0.8898
              D22G012      17.3473          10     0.0670     0.5122
              D22G013      38.8062          21     0.0104     0.0390
              D22G014      17.2802          15     0.3024     0.4651
```

Example 2.2. Computing Linkage Disequilibrium Measures for SNP Data

The following data set contains 44 individuals' genotypes at five SNPs.

```
data snps;
   input s1-s10;
   datalines;
2 2 2 1 2 1 1 1 2 2
2 2 2 2 2 1 1 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 . . 1 1 2 2
2 2 2 2 1 2 1 2 2 2
2 2 2 2 . . 2 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 . . 2 1 2 2
2 2 2 2 1 1 1 1 2 2
2 2 1 1 2 2 2 1 2 2
2 2 2 1 2 2 2 1 2 2
2 2 2 2 1 1 1 1 2 2
2 2 2 1 2 2 2 2 2 2
2 2 2 2 2 2 1 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 1 2 2
2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 1 1 1 2 2
2 2 2 2 1 1 2 1 2 2
2 2 2 2 2 1 1 1 2 2
2 2 2 2 2 1 2 2 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 2 1 2 2 2 2
2 2 2 2 2 1 2 2 2 2
2 2 2 2 2 2 1 1 2 2
2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 1 1 2 2
2 2 2 2 2 2 1 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 2 2 . . 2 2
2 2 2 1 2 2 2 1 2 2
2 2 2 2 2 2 2 1 2 2
2 2 2 2 2 1 1 1 2 2
2 2 2 2 2 2 1 1 2 2
2 2 2 2 2 1 2 1 2 2
2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 1 2 2
2 2 2 2 2 2 2 1 2 2
;
```

Now an analysis using PROC ALLELE can be performed as follows:

```
proc allele data=snps prefix=SNP nofreq haplo=est corrcoeff dprime yulesq;
   var s1-s10;
run;
```

This analysis produces summary statistics of the five SNPs as well as the Linkage Disequilibrium Measures table, which contains estimated two-locus haplotype frequencies and disequilibrium coefficients, and the linkage disequilibrium measures r, D', and Q. The allele and genotype frequency output tables are suppressed with the NOFREQ option.

The results from the analysis are as follows. Note the names of the markers that are used.

Output 2.2.1. Summary of SNPs for the ALLELE Procedure

```
                              The ALLELE Procedure

                                Marker Summary

                                                    -------Test for HWE-------
        Number   Number
            of       of             Hetero-    Allelic        Chi-           Pr >
Locus    Indiv  Alleles      PIC  zygosity  Diversity      Square      DF   ChiSq

SNP1        44        1  0.0000    0.0000     0.0000      0.0000       0     .
SNP2        44        2  0.1190    0.0909     0.1271      3.5627       1  0.0591
SNP3        41        2  0.3283    0.4390     0.4140      0.1493       1  0.6992
SNP4        43        2  0.3728    0.4884     0.4957      0.0093       1  0.9231
SNP5        44        1  0.0000    0.0000     0.0000      0.0000       0     .
```

There are two SNPs that have only one allele appearing in the data.

Output 2.2.2. Linkage Disequilibrium Measures for SNPs Using the ALLELE Procedure

```
                         Linkage Disequilibrium Measures

                                                 LD      Corr  Lewontin's    Yule's
Locus1  Locus2  Haplotype  Frequency          Coeff     Coeff          D'         Q

SNP1    SNP2    2-1           0.0682        -0.0000     .           .         .
SNP1    SNP2    2-2           0.9318        -0.0000     .           .         .
SNP1    SNP3    2-1           0.2927        -0.0000     .           .         .
SNP1    SNP3    2-2           0.7073        -0.0000     .           .         .
SNP1    SNP4    2-1           0.5465        -0.0000     .           .         .
SNP1    SNP4    2-2           0.4535        -0.0000     .           .         .
SNP1    SNP5    2-2           1.0000         0.0000     .           .         .
SNP2    SNP3    1-2           0.0732         0.0214   0.1807      1.0000    1.0000
SNP2    SNP3    2-1           0.2927         0.0214   0.1807      1.0000    1.0000
SNP2    SNP3    2-2           0.6341        -0.0214  -0.1807     -1.0000   -1.0000
SNP2    SNP4    1-1           0.0331        -0.0050  -0.0398     -0.1322   -0.1546
SNP2    SNP4    1-2           0.0367         0.0050   0.0398      0.1322    0.1546
SNP2    SNP4    2-1           0.5134         0.0050   0.0398      0.1322    0.1546
SNP2    SNP4    2-2           0.4168        -0.0050  -0.0398     -0.1322   -0.1546
SNP2    SNP5    1-2           0.0682        -0.0000     .           .         .
SNP2    SNP5    2-2           0.9318        -0.0000     .           .         .
SNP3    SNP4    1-1           0.2221         0.0608   0.2661      0.4382    0.5529
SNP3    SNP4    1-2           0.0779        -0.0608  -0.2661     -0.4382   -0.5529
SNP3    SNP4    2-1           0.3154        -0.0608  -0.2661     -0.4382   -0.5529
SNP3    SNP4    2-2           0.3846         0.0608   0.2661      0.4382    0.5529
SNP3    SNP5    1-2           0.2927        -0.0000     .           .         .
SNP3    SNP5    2-2           0.7073        -0.0000     .           .         .
SNP4    SNP5    1-2           0.5465        -0.0000     .           .         .
SNP4    SNP5    2-2           0.4535        -0.0000     .           .         .
```

In the preceding table, the values for the linkage disequilibrium measures are missing for several haplotypes; this occurs when there is only one allele at one of the markers contained in the haplotype, and thus the denominators for these measures are zero. Also note that when the markers are biallelic, the gametic disequilibria have the same absolute values for all four possible haplotypes.

References

Botstein, D., White, R.L., Skolnick, M., and Davis, R.W. (1980), "Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms," *American Journal of Human Genetics,* 32, 314–331.

Devlin, B. and Risch, N. (1995), "A Comparison of Linkage Disequilibrium Measures for Fine-Scale Mapping," *Genomics,* 29, 311–322.

Guo, S.W. and Thompson, E.A. (1992), "Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles," *Biometrics,* 48, 361–372.

Weir, B.S. (1979), "Inferences about Linkage Disequilibrium," *Biometrics,* 35, 235–254.

Weir, B.S. (1996), *Genetic Data Analysis II,* Sunderland, MA: Sinauer Associates, Inc.

Weir, B.S. and Cockerham, C.C. (1979), "Estimation of Linkage Disequilibrium in Randomly Mating Populations," *Heredity,* 42, 105–111.

Wijsman, E.M., Almasy, L., Amos, C.I., Borecki, I., Falk, C.T., King, T.M., Martinez, M.M., Meyers, D., Neuman, R., Olson, J.M., Rich, S., Spence, M.A., Thomas, D.C., Vieland, V.J., Witte, J.S., and MacCluer, J.W. (2001), "Analysis of Complex Genetic Traits: Applications to Asthma and Simulated Data," *Genetic Epidemiology,* 21, S1–S853.

Zaykin, D., Zhivotovsky, L., and Weir, B.S. (1995), "Exact Tests for Association between Alleles at Arbitrary Numbers of Loci," *Genetica,* 96, 169–178.

Chapter 3
The CASECONTROL Procedure

Chapter Contents

Chapter 3
The CASECONTROL Procedure

Overview

Marker information can be used to help locate the genes that affect susceptibility to a disease. The CASECONTROL procedure is designed for the interpretation of marker data when random samples are available from the populations of unrelated individuals who are either affected or unaffected by the disease. Several tests are available in PROC CASECONTROL that compare marker allele and/or genotype frequencies in the two populations, with frequency differences indicating an association of the marker with the disease. Although such an association may point to the proximity of the marker and disease genes in the genome, it may also reflect population structure, so care is needed in interpreting the results; association does not necessarily imply linkage.

The three chi-square tests available for testing case-control genotypic data are the genotype case-control test, which tests for dominant allele effects on the disease penetrance, and the allele case-control test and linear trend test, which test for additive allele effects on the disease penetrance. Since the allele case-control test requires the assumption of Hardy-Weinberg equilibrium (HWE), it may be desirable to run the ALLELE procedure on the data to perform the HWE test on each marker (see Chapter 2, “The ALLELE Procedure,” for more information) prior to applying PROC CASECONTROL.

Getting Started

Example

Here are some sample SNP data on which the three case-control tests can be performed using PROC CASECONTROL:

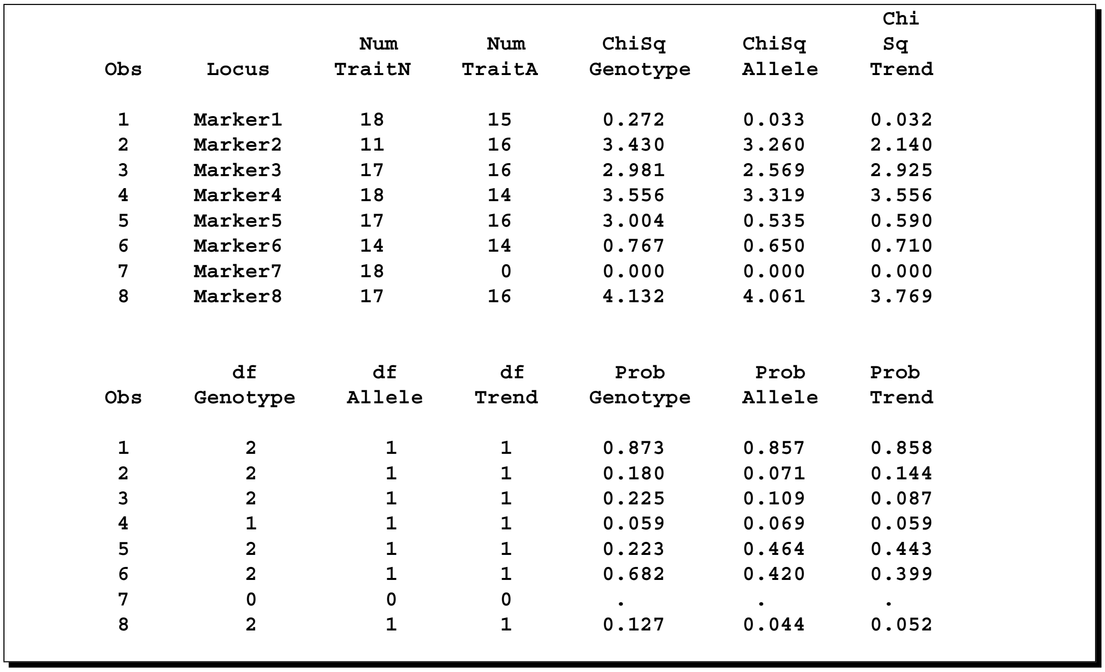
```
data cc;
   input affected $ m1-m16;
   datalines;
 N  1 1 2 2 2 2 2 1 2 1 2 2 1 1 2 2
 N  1 1 1 1 2 2 1 1 2 1 2 1 1 1 1 1
 N  2 1 1 1 2 1 1 1 2 2 1 1 1 1 1 1
 N  2 2 2 1 2 2 1 1 2 2 2 1 1 1 2 2
 N  1 1 1 1 2 2 2 1 1 1 1 1 2 1 . .
 N  2 1 1 1 2 1 1 1 2 1 2 1 1 1 2 1
 N  1 1 1 1 2 2 1 1 2 2 2 2 2 1 2 2
 N  2 2 1 1 2 1 2 1 2 2 2 1 1 1 2 1
 N  2 1 1 1 2 2 2 1 2 1 . . 1 1 2 1
 N  2 1 1 1 2 1 1 1 2 2 1 1 1 1 1 1
 N  2 1 2 2 . . 1 1 2 1 1 1 1 1 1 1
```

```
N  2 2 . . 2 1 1 1 2 1 2 1 1 1 2 1
N  2 1 . . 2 2 1 1 2 2 1 1 1 1 2 1
N  2 1 . . 2 2 1 1 2 1 . . 2 1 1 1
N  2 2 . . 2 2 1 1 . . 2 1 1 1 2 1
N  1 1 . . 2 2 1 1 1 1 2 1 1 1 2 1
N  1 1 . . 2 2 1 1 1 1 . . 1 1 2 1
N  2 1 . . 2 2 1 1 1 1 . . 2 1 2 1
A  2 1 2 1 2 1 1 1 1 1 2 1 . . 2 1
A  2 1 2 1 2 2 1 1 2 1 1 1 . . 1 1
A  2 2 2 1 2 2 1 1 2 2 . . . . 2 1
A  2 1 2 2 2 1 1 1 2 1 2 1 . . 2 2
A  . . 2 2 2 1 . . 1 1 2 2 . . 2 1
A  1 1 1 1 2 1 1 1 2 1 1 1 . . 2 2
A  2 1 1 1 2 2 1 1 1 1 2 1 . . 2 1
A  2 1 2 2 2 2 1 1 2 2 . . . . 2 2
A  2 1 1 1 2 2 1 1 2 1 2 1 . . 1 1
A  2 1 2 2 2 1 1 1 2 1 2 1 . . 2 2
A  1 1 1 1 2 2 1 1 2 1 2 1 . . 2 2
A  2 1 2 1 2 1 1 1 2 1 2 2 . . 2 1
A  2 2 2 2 1 1 1 1 2 1 2 1 . . 2 2
A  1 1 1 1 2 1 . . 2 1 2 2 . . 2 2
A  1 1 2 1 2 1 1 1 2 1 2 1 . . 2 2
A  2 2 1 1 2 2 1 1 2 1 1 1 . . 2 1
;
```

The following SAS code can be used to perform the analysis:

```
proc casecontrol data=cc prefix=Marker;
   var m1-m16;
   trait affected;
run;

proc print heading=h;
run;
```

All three case-control tests are performed by default. The output data set created by default appears as follows:



```
                                                                       Chi
                            Num       Num       ChiSq       ChiSq       Sq
 Obs       Locus          TraitN    TraitA     Genotype    Allele     Trend

   1      Marker1           18        15        0.272       0.033     0.032
   2      Marker2           11        16        3.430       3.260     2.140
   3      Marker3           17        16        2.981       2.569     2.925
   4      Marker4           18        14        3.556       3.319     3.556
   5      Marker5           17        16        3.004       0.535     0.590
   6      Marker6           14        14        0.767       0.650     0.710
   7      Marker7           18         0        0.000       0.000     0.000
   8      Marker8           17        16        4.132       4.061     3.769


              df          df         df         Prob        Prob      Prob
 Obs      Genotype      Allele     Trend     Genotype     Allele     Trend

   1          2            1          1        0.873       0.857     0.858
   2          2            1          1        0.180       0.071     0.144
   3          2            1          1        0.225       0.109     0.087
   4          1            1          1        0.059       0.069     0.059
   5          2            1          1        0.223       0.464     0.443
   6          2            1          1        0.682       0.420     0.399
   7          0            0          0          .           .         .
   8          2            1          1        0.127       0.044     0.052
```

Figure 3.1. Statistics for Case-Control Tests

Figure 3.1 displays the statistics for the three tests. The genotype case-control statistic has more degrees of freedom than the other two because it is testing for both dominance genotypic effects and additive allelic effects, while the other statistics are testing for the significant additive effects alone. Using the standard significance level of 0.05, none of the p-values, shown in the last three columns, would be considered significant since they are all above this significance level. Thus, you would conclude that none of the markers show a significant association with the binary trait. The p-values for Marker7 are missing because the genotypes of all the affected individuals are missing at that marker.

Syntax

The following statements are available in PROC CASECONTROL.

PROC CASECONTROL < *options* > ;
  BY *variables* ;
  TRAIT *variable* ;
  VAR *variables* ;

Items within angle brackets (< >) are optional, and statements following the PROC CASECONTROL statement can appear in any order. The TRAIT and VAR statements are required. The syntax of each statement is described in the following section in alphabetical order after the description of the PROC CASECONTROL statement.

PROC CASECONTROL Statement

PROC CASECONTROL < *options* > **;**

You can specify the following options in the PROC CASECONTROL statement.

ALLELE

requests that the allele case-control test be performed. If none of the three test options (ALLELE, GENOTYPE, or TREND) are specified, then all three tests are performed by default.

DATA=*SAS-data-set*

names the input SAS data set to be used by PROC CASECONTROL. The default is to use the most recently created data set.

DELIMITER=*'string'*

indicates the string that is used to separate the two alleles that comprise the genotypes contained in the variables specified in the VAR statement. This option is ignored if GENOCOL is not specified.

GENOCOL

indicates that columns specified in the VAR statement contain genotypes instead of alleles. When this option is specified, there is one column per marker. The genotypes must consist of the two alleles separated by a delimiter.

GENOTYPE

requests that the genotype case-control test be performed. If none of the three test options (ALLELE, GENOTYPE, or TREND) are specified, then all three tests are performed by default.

Experimental

INDIVIDUAL=*variable*

INDIV=*variable*

specifies the individual ID variable when using the experimental TALL option. This variable may be character or numeric.

Experimental

MARKER=*variable*

specifies the marker ID variable when using the TALL option. This variable contains the names of the markers that are used in all output and may be character or numeric.

NDATA=*SAS-data-set*

names the input SAS data set containing names, or identifiers, for the markers used in the output. There must be a NAME variable in this data set, which should contain the same number of rows as there are markers in the input data set specified in the DATA= option. When there are fewer rows than there are markers, markers without a name are named using the PREFIX= option. Likewise, if there is no NDATA= data set specified, the PREFIX= option is used. Note that this data set is ignored if the experimental TALL option is specified in the PROC CASECONTROL statement. In that case, the marker variable names are taken from the marker ID variable specified in the MARKER= option.

NULLSNPS=(*variable list*)

names the markers to be used in calculating the variance inflation factor for genomic control that is applied to the chi-square statistic(s) from the trend test. Only biallelic markers that are listed are used. Note that if GENOCOL is specified, there should be one variable for each marker listed; otherwise, there should be two variables per marker. By default, if VIF is specified in the PROC CASECONTROL statement, all biallelic markers listed in the VAR statement are used. This option must be specified if both the VIF option and the PERMS= option are used, otherwise the variance inflation factor is not applied. This option is ignored if the VIF option is not specified or if the experimental TALL option is used.

OUTSTAT=*SAS-data-set*

names the output SAS data set containing counts for the two trait values, the chi-square statistics, degrees of freedom, and p-values for the tests performed. When this option is omitted, an output data set is created by default and named according to the DATA*n* convention.

PERMS=*number*

indicates that Monte Carlo estimates of exact p-values for the case-control tests should be calculated instead of the p-values from the asymptotic χ^2 distribution. In each of the *number* permutation samples, the trait values are permuted among the individuals in the sample. Large values of *number* (10,000 or more) are usually recommended for accuracy, but long execution times may result, particularly with large data sets. When this option is omitted, no permutations are performed and p-values from the asymptotic χ^2 distribution are reported.

PREFIX=*prefix*

specifies a prefix to use in constructing names for marker variables in all output. For example, if PREFIX=VAR, the names of the variables are VAR1, VAR2, ..., VAR*n*. Note that this option is ignored when the NDATA= option is specified, unless there are fewer names in the NDATA data set than there are markers; it is also ignored if the experimental TALL option is specified, in which case the marker variable names are taken from the marker ID variable specified in the MARKER= option. Otherwise, if this option is omitted, PREFIX=M is the default when variables contain alleles; if GENOCOL is specified, then the names of the variables specified in the VAR statement are used as the marker names.

SEED=*number*

specifies the initial seed for the random number generator used for permuting the data to calculate estimates of exact p-values. This option is ignored if PERMS= is not specified. The value for *number* must be an integer; the computer clock time is used if the option is omitted or an integer less than or equal to 0 is specified. For more details about seed values, refer to *SAS Language Reference: Concepts*.

TALL *Experimental*

indicates that the input data set is of an alternative format. This tall-skinny format contains the following columns: two containing marker alleles (or one containing marker genotypes if GENOCOL is specified), one for the marker identifier, and one for the individual identifier. The experimental MARKER= and INDIV= options must also be specified for this option to be in effect. Note that when this option is used, the

DATA= data set must first be sorted by any BY variables, then sorted by the marker ID variable, then the individual ID variable.

TREND

requests that the linear trend test for allelic effects be performed. If none of the three test options (ALLELE, GENOTYPE, or TREND) are specified, then all three tests are performed by default.

VIF

specifies that the variance inflation factor λ should be applied to the trend chi-square statistic for genomic control. This adjustment is applied only when the trend test is performed and to markers in the VAR statement that are biallelic.

BY Statement

BY *variables* **;**

You can specify a BY statement with PROC CASECONTROL to obtain separate analyses on observations in groups defined by the BY variables. When a BY statement appears, the procedure expects the input data set to be sorted in order of the BY variables. The *variables* are one or more variables in the input data set.

If your input data set is not sorted in ascending order, use one of the following alternatives:

- Sort the data using the SORT procedure with a similar BY statement.
- Specify the BY statement option NOTSORTED or DESCENDING in the BY statement for the CASECONTROL procedure. The NOTSORTED option does not mean that the data are unsorted but rather that the data are arranged in groups (according to values of the BY variables) and that these groups are not necessarily in alphabetical or increasing numeric order.
- Create an index on the BY variables using the DATASETS procedure (in base SAS software).

For more information on the BY statement, refer to the discussion in *SAS Language Reference: Concepts*. For more information on the DATASETS procedure, refer to the discussion in the *SAS Procedures Guide*.

TRAIT Statement

TRAIT *variable* **;**

The TRAIT statement identifies a binary variable indicating which individuals are cases and which are controls or a binary variable representing a dichotomous trait. This variable can be character or numeric, but must have only two nonmissing levels.

VAR Statement

VAR *variables* **;**

The VAR statement identifies the variables containing either the marker alleles, or the marker genotypes if GENOCOL is specified. The following number of variables should be specified in this statement for a data set containing m markers according to whether the options GENOCOL and TALL are used:

- When both GENOCOL and TALL are specified, there should be 1 variable named containing marker genotypes
- When only TALL is specified, there should be 2 variables named containing marker alleles
- When only GENOCOL is specified, there should be m variables named, one for each marker containing marker genotypes
- When neither option is specified, there should be $2m$ variables named, two for each marker containing marker alleles

All variables specified must be of the same type, either character or numeric.

Details

Statistical Computations

Biallelic Markers

PROC CASECONTROL offers three statistics to test for an association between a biallelic marker and a binary variable, typically affection status of a particular disease. Table 3.1 displays the quantities that are used for the three case-control tests for biallelic markers (Sasieni 1997).

Table 3.1. Genotype Distribution for Case-Control Sample

	Number of M_1 alleles			
	0	1	2	Total
Case	r_0	r_1	r_2	R
Control	s_0	s_1	s_2	S
Total	n_0	n_1	n_2	N

The three statistical methods for testing a marker for association with a disease locus are Armitage's trend test (1955), the allele case-control test, and the genotype case-control test. The trend test and allele case-control test are most useful when there is an additive allele effect on the disease susceptibility. When Hardy-Weinberg equilibrium (HWE) holds in the combined sample of cases and controls, these statistics are approximately equal and have an asymptotic χ^2_1 distribution. However, if the assumption of HWE in the combined sample is violated, then the variance for the allele case-control statistic is incorrect; only the trend test remains valid under this violation. The statistics for the trend and allele case-control test, respectively, are given by Sasieni (1997) as

$$\begin{aligned} X_T^2 &= \frac{N[N(r_1+2r_2)-R(n_1+2n_2)]^2}{R(N-R)[N(n_1+4n_2)-(n_1+2n_2)^2]} \\ X_A^2 &= \frac{2N[2N(r_1+2r_2)-2R(n_1+2n_2)]^2}{(2R)2(N-R)[2N(n_1+2n_2)-(n_1+2n_2)^2]} \end{aligned}$$

Devlin and Roeder (1999) describe a genomic control method that adjusts the trend test statistic for correlation between alleles from members of the same subpopulation. Assuming the variance inflation factor λ is constant across the genome, it can be estimated by $\hat{\lambda} = \max([\text{median}(X_1, ..., X_m)/0.675]^2, 1)$, where $X_i = X_T$ for the ith biallelic marker, $i = 1, ..., m$ (Devlin and Roeder 1999; Bacanu, Devlin, and Roeder 2000). The adjusted trend statistic, $X_{T_a}^2 = X_T^2/\hat{\lambda}$, is approximately distributed as χ_1^2. This variance correction is made to biallelic markers when the VIF option is specified in the PROC statement. By default, any biallelic markers that are specified in the VAR statement are used in computing $\hat{\lambda}$. Alternatively, the NULLSNPS= option can be used to specify biallelic markers other than those in the VAR statement to be used to calculate $\hat{\lambda}$. This allows markers that are assumed to have no effect on disease susceptibility or to not be in linkage disequilibrium with a disease-susceptibility locus to be used in calculating the inflation factor (Bacanu, Devlin, and Roeder 2000).

If dominance effects of alleles are also suspected to contribute to disease susceptibility, the genotype case-control test can be used. The standard 2×3 contingency table analysis is used to form the χ_2^2 statistic for the genotype case-control test as

$$X_G^2 = \sum_{i=0}^{2} \left[\frac{(Nr_i - Rn_i)^2}{NRn_i} + \frac{(Ns_i - Sn_i)^2}{NSn_i} \right]$$

which tests for both additive and dominance (nonadditive) allelic effects (Nielsen and Weir 1999).

Multiallelic Markers

When there are multiple alleles of interest at a marker, the same three tests can be performed, except that Devlin and Roeder's genomic control adjustment is not applied when there are any markers with more than two alleles. To construct the test statistic for the multiallelic trend test for a marker with k alleles (Slager and Schaid 2001), the $p \times (k-1)$ matrix $\mathbf{X}$ is created such that each element X_{iu} represents the number of times the M_u allele appears in the ith genotype, $i = 1, ..., p$ and $u = 1, ..., k-1$, where $p = k(k+1)/2$, the number of possible genotypes. Vectors $\mathbf{r}$ and $\mathbf{s}$ of length p contain the genotype counts for the cases and controls respectively, and $\phi = R/N$, the proportion of cases in the sample. The multiallelic trend test statistic can then be expressed as $\mathbf{U}'[\text{Var}(\mathbf{U})]^{-1}\mathbf{U}$, where the vector $\mathbf{U} = \mathbf{X}'[(1-\phi)\mathbf{r} - \phi\mathbf{s}]$. Var($\mathbf{U}$) is calculated under the assumption of independent (or unrelated) subjects in the sample using Var($\mathbf{r}$) and Var($\mathbf{s}$). These matrices contain elements $\sigma_{ii} = Rn_i(N-n_i)/N^2$

and $\sigma_{ij} = -Rn_in_j/N^2$, where $i, j = 1, ..., p$ (the R is replaced by S for Var($\mathbf{s}$)). This statistic has an asymptotic χ^2_{k-1} distribution.

Another way to test for additive allele effects at the disease or trait locus is the allele case-control test, executed using a contingency table analysis similar to the genotype case-control test described in the preceding section, assuming HWE (Nielsen and Weir 1999). For a marker with k alleles, a $2 \times k$ contingency table is formed with one row for cases, one for controls, and a column for each allele. The χ^2_{k-1} statistic is formed by summing $(O - E)^2/E$ over all cells in the table, where O is the observed count for the cell and E is the expected count, the cell's column total multiplied by R/N (or S/N) for a cell in the case (or control) row.

The genotypic case-control test statistic is calculated in a similar manner, with columns now representing the p observed genotype classes instead of alleles. Significance of this test statistic using the χ^2_{p-1} distribution indicates dominance and/or additive allelic effects on the disease or trait (Nielsen and Weir 1999).

Permutation Tests

By default, the p-values from the χ^2 distribution with the appropriate degrees of freedom are reported for all three case-control tests. However, if the PERMS= option is specified in the PROC CASECONTROL statement, then Monte Carlo estimates of exact p-values are computed instead using the permutation procedure. New samples of individuals are formed by permuting the trait value of the individuals in the sample. If p permutations are performed, the exact p-value is calculated as the proportion of times the chi-square statistic from one of the p new samples is equal to or exceeds the chi-square statistic from the original sample (Westfall and Young 1993).

Missing Values

An individual's genotype for a marker is considered missing if at least one of the alleles at the marker is missing. Any missing genotypes are excluded from all calculations. However, the individual's nonmissing genotypes at other loci can be used as part of the calculations. If an individual has a missing trait value, then that individual is excluded from all calculations.

OUTSTAT= Data Set

The output data set specified in the OUTSTAT= option of the PROC CASECONTROL statement contains the following variables:

- the BY variables, if any
- Locus
- the counts of genotyped individuals for the two values of the TRAIT variable: NumTrait1 and NumTrait2, where 1 and 2 are replaced by the values of the TRAIT variable
- the chi-square statistic for each test performed: ChiSqAllele, ChiSqGenotype, and ChiSqTrend

- the degrees of freedom for each test performed: dfAllele, dfGenotype, and dfTrend
- the p-value for each test performed: ProbAllele, ProbGenotype, and ProbTrend

Examples

Example 3.1. Performing Case-Control Tests on Multiallelic Markers

The following data are taken from GAW9 (Hodge 1995). A sample of 60 founders was taken from 200 nuclear families, 30 affected with a disease and 30 unaffected. Each founder was genotyped at two marker loci.

```
data founders;
   input id disease a1-a4 @@;
   datalines;
4   1 6 4 3 7  17  2 4 7 2 7
39  2 6 8 7 7  41  2 4 4 4 7
46  1 8 4 1 5  50  2 4 2 3 7
54  2 4 8 7 6  56  2 7 4 7 7
62  2 4 1 7 3  69  2 6 8 2 7
79  1 6 6 8 7  80  2 6 4 7 3
83  2 8 4 2 7  85  1 5 6 6 2
95  1 3 2 3 7  101 1 4 6 7 7
106 1 2 1 7 2  107 1 1 2 7 7
115 2 4 2 7 5  116 1 4 1 7 3
120 2 1 6 2 7  123 2 4 4 7 2
130 1 5 2 3 7  133 1 8 6 3 6
134 1 8 4 2 2  139 2 6 4 7 6
142 2 3 6 7 7  151 1 4 6 4 3
152 1 6 7 6 7  153 1 5 1 7 6
154 1 4 6 6 6  168 1 1 4 3 7
178 2 4 1 7 1  187 1 1 8 1 2
189 2 6 4 5 7  190 2 4 4 3 7
195 2 4 4 7 2  207 2 1 6 7 7
216 1 7 4 1 5  222 2 4 2 7 3
225 2 8 7 7 6  234 1 6 4 2 2
244 1 4 4 7 6  249 2 6 8 7 2
263 1 8 2 3 7  267 2 2 2 2 7
276 2 1 6 7 1  284 2 4 8 2 2
286 1 8 8 2 1  289 1 2 6 6 3
290 1 2 4 5 7  294 2 1 8 6 7
297 2 5 4 7 6  313 1 1 7 7 2
337 1 2 6 7 6  366 2 2 2 7 7
368 2 3 1 7 2  381 1 6 4 5 3
384 1 6 2 2 7  396 1 4 5 7 2
;
```

The multiallelic versions of the association tests are performed since each marker has more than two alleles. The following code invokes the three case-control tests to find out whether there is a significant association between either of the markers and disease status. Note that the same output could be produced by omitting the three tests, ALLELE, GENOTYPE, and TREND, from the PROC CASECONTROL statement.

```
proc casecontrol data=founders genotype allele trend;
   trait disease;
   var a1-a4;
run;

proc print noobs heading=h;
run;
```

An output data set is created by default, and the output from the PRINT procedure is displayed in Output 3.1.1.

Output 3.1.1. Output Data Set from PROC CASECONTROL for Multiallelic Markers

```
                Num       Num      ChiSq     ChiSq     ChiSq       df
   Locus      Trait1    Trait2    Genotype   Allele    Trend    Genotype

    M1          30        30       27.333     4.441     5.039       24
    M2          30        30       18.077     8.772    13.244       15

     df         df        Prob      Prob      Prob
   Allele     Trend    Genotype    Allele     Trend

      7         7       0.2892    0.7278    0.6552
      7         7       0.2586    0.2694    0.0664
```

This analysis finds no significant association between disease status and either of the markers. Suppose, however, that allele 7 of the second marker had been identified by previous studies as an allele of interest for this particular disease, and thus there is concern that its effect is swamped by the other seven alleles. The data set can be modified so that the second marker is considered a biallelic marker with alleles 7 and "not 7."

```
data marker2;
   set founders;
   if a3 ne 7 then a3=1;
   if a4 ne 7 then a4=1;
   keep id a3 a4 disease;
```

Now all three tests can be performed on the marker in the new data set.

```
proc casecontrol data=marker2;
   trait disease;
   var a3 a4;
run;

proc print noobs heading=h;
run;
```

PROC CASECONTROL performs all three tests by default since none were specified. The output data set for this analysis is displayed in Output 3.1.2.

Output 3.1.2. Output Data Set from PROC CASECONTROL for a Biallelic Marker

```
                 Num        Num        ChiSq      ChiSq      ChiSq        df
   Locus       Trait1     Trait2     Genotype    Allele      Trend     Genotype

    M1           30         30        12.193      6.599     10.103         2

     df          df        Prob        Prob        Prob
   Allele      Trend     Genotype     Allele      Trend

      1           1       0.0023      0.0102     0.0015
```

With just the single allele of interest, there is now a significant association (using a significance level of $\alpha = 0.05$) according to all three case-control tests between the marker (specifically, allele 7) and disease status. Note that the allele and trend tests, both of which are testing for additive allele effects, produce quite different p-values, which could be an indication that HWE does not hold for allele 7. This is in fact the case, which can be checked by running the ALLELE procedure on data set marker2 to test for HWE (see Chapter 2, "The ALLELE Procedure," for more information). The excess of heterozygotes forces X_A^2 to be smaller than X_T^2, and only X_T^2 remains a valid chi-square statistic under the HWE violation.

Example 3.2. Analyzing Data in the Tall-Skinny Format

This example demonstrates how data in the tall-skinny format can be analyzed using PROC CASECONTROL with the experimental options TALL, MARKER=, and INDIV=. Here, the same data that were used in the "Getting Started" example are used, but in this alternative format.

```
data talldata;
   input affected $ id snpname $ allele1 allele2;
   datalines;
 N  1 Marker1 1 1
 N  2 Marker1 1 1
 N  3 Marker1 2 1
 N  4 Marker1 2 2
 N  5 Marker1 1 1
 N  6 Marker1 2 1
 N  7 Marker1 1 1
 N  8 Marker1 2 2
 N  9 Marker1 2 1
 N 10 Marker1 2 1
 N 11 Marker1 2 1
 N 12 Marker1 2 2
 N 13 Marker1 2 1
 N 14 Marker1 2 1
 N 15 Marker1 2 2
 N 16 Marker1 1 1
 N 17 Marker1 1 1
 N 18 Marker1 2 1
 A 19 Marker1 2 1
 A 20 Marker1 2 1
 A 21 Marker1 2 2
 A 22 Marker1 1 2
 A 24 Marker1 1 1
 A 25 Marker1 2 1
 A 26 Marker1 2 1
 A 27 Marker1 2 1
 A 28 Marker1 2 1
 A 29 Marker1 1 1
 A 30 Marker1 2 1
 A 31 Marker1 2 2
 A 32 Marker1 1 1
 A 33 Marker1 1 1
 A 34 Marker1 2 2
 N  1 Marker2 2 2
 N  2 Marker2 1 1

   ...

 A 28 Marker8 2 2
 A 29 Marker8 2 2
 A 30 Marker8 1 2
 A 31 Marker8 2 2
 A 32 Marker8 2 2
 A 33 Marker8 2 2
 A 34 Marker8 1 2
;
```

Note how all marker alleles are contained in two columns, and there are identifiers for the markers and individuals sampled. The data set is first sorted by the marker ID, then the individual ID. One advantage of this data format is there is no restriction on the number of markers analyzed since, unlike the columns, there is no limit on the number of rows in a SAS data set. The following code can be used to analyze this data:

```
proc casecontrol data=talldata tall marker=snpname indiv=id;
   var allele1 allele2;
   trait affected;
run;

proc print;
run;
```

Applying this code to the data in this format produces the same output shown in the "Getting Started" example, Figure 3.1.

References

Armitage, P. (1955), “Tests for Linear Trends in Proportions and Frequencies,” *Biometrics,* 11, 375–386.

Bacanu, S-A., Devlin, B., and Roeder, K. (2000), “The Power of Genomic Control,” *American Journal of Human Genetics,* 66, 1933–1944.

Devlin, B. and Roeder, K. (1999), “Genomic Control for Association Studies,” *Biometrics,* 55, 997–1004.

Hodge, S.E. (1995), “An Oligogenic Disease Displaying Weak Marker Associations: A Summary of Contributions to Problem 1 of GAW9,” *Genetic Epidemiology,* 12, 545–554.

Nielsen, D.M. and Weir, B.S. (1999), “A Classical Setting for Associations between Markers and Loci Affecting Quantitative Traits,” *Genetic Research,* 74, 271–277.

Sasieni, P.D. (1997), “From Genotypes to Genes: Doubling the Sample Size,” *Biometrics,* 53, 1253–1261.

Slager, S.L. and Schaid, D.J. (2001), “Evaluation of Candidate Genes in Case-Control Studies: A Statistical Method to Account for Related Subjects,” *American Journal of Human Genetics,* 68, 1457–1462.

Westfall, P.H. and Young, S.S. (1993), *Resampling-based Multiple Testing,* New York: John Wiley & Sons, Inc.

Chapter 4
The FAMILY Procedure

Chapter Contents

Chapter 4
The FAMILY Procedure

Overview

Family genotype data, though more difficult to collect, often provide a more effective way of testing markers for association with disease status than case-control data. Case-control data may uncover significant associations between markers and a disease that could be caused by factors other than linkage, such as population structure. Analyzing family data using the FAMILY procedure ensures that any significant associations found between a marker and disease status are due to linkage between the marker and disease locus. This is accomplished by using the transmission/disequilibrium test (TDT) and several variations of it that can accommodate different types of family data. One type of family consists of parents, at least one heterozygous, and an affected child who have all been genotyped. This family structure is suitable for the original TDT. Families containing at least one affected and one unaffected sibling from a sibship that have both been genotyped can be analyzed using the sibling tests: the sib TDT (S-TDT) or the nonparametric sibling disequilibrium test (SDT). Both types of families can be jointly analyzed using the combined versions of the S-TDT and SDT and the reconstruction-combined TDT (RC-TDT). The RC-TDT can additionally accommodate families with no unaffected children and missing parental genotypes in certain situations.

Getting Started

Example

The following example demonstrates how you can use PROC FAMILY to perform one of several family-based tests, the TDT. You have collected the following family genotypic data that you input into a SAS data set:

```
data example;
   input ped indiv father mother disease (a1-a4)($);
   datalines;
 1   1  0   0 1 a b a c
 1   2  0   0 1 c c a d
 1 101  1   2 1 a c a d
 1 102  1   2 1 b c a d
 1 103  1   2 1 a c c a
 1 104  1   2 2 b c a a
 2   3  0   0 1 e e f g
 2   4  0   0 1 d e g a
 2 105  3   4 1 e d f a
 2 106  3   4 2 e e g a
 3   5  0   0 1 d a a c
```

```
   3   6  0  0 1 e e c a
   3 107  5  6 2 a e a a
   4   7  0  0 1 f b a g
   4   8  0  0 1 c e h g
   4 108  7  8 2 b e a g
   4 109  7  8 1 f c g g
   4 110  7  8 1 b c a g
   4 111  7  8 1 b c a h
   5   9  0  0 1 a f d c
   5  10  0  0 1 h d c h
   5 112  9 10 2 a d d c
   5 113  9 10 1 f d d c
   6  11  0  0 1 b e c g
   6  12  0  0 1 d f a g
   6 114 11 12 2 b f c a
   6 115 11 12 1 b d g a
   7  13  0  0 1 e d c c
   7  14  0  0 1 e h d a
   7 116 13 14 1 e h c a
   7 117 13 14 2 d e c a
   7 118 13 14 1 d h c d
   7 119 13 14 1 d h c d
  ;
```

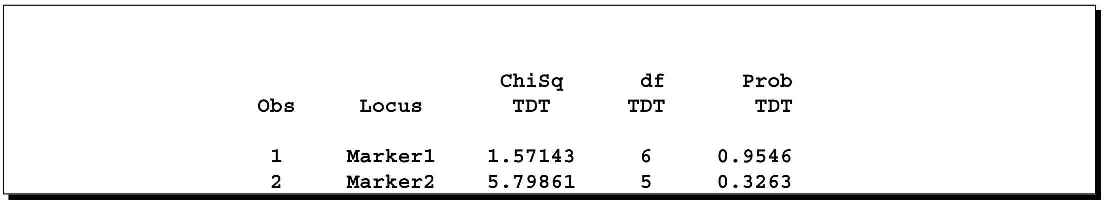
The first column of the data set contains the pedigree ID, followed by an individual ID, and the two parental IDs. The fifth column is a variable representing affection status of a disease. The last four columns of this data set contain the two alleles at each of two markers for each individual. Since there are no missing parental genotypes in this data set, the TDT is a reasonable test to perform in order to determine if either of the two markers is significantly linked to the disease locus whose location you are trying to pinpoint. Furthermore, close inspection of the data reveals that there is only one affected child (which corresponds to a value of “2” for the disease affection variable) per each family. Thus, the TDT is also a valid test for association with the disease locus. To perform the analysis, you would use the following statements:

```
proc family data=example prefix=Marker outstat=stats tdt contcorr;
   id ped indiv father mother;
   trait disease / affected=2;
   var a1-a4;
run;

proc print data=stats;
   format ProbTDT pvalue6.5;
run;
```

This code creates an output data set stats, which contains the chi-square statistic, degrees of freedom, and p-value for testing each marker for linkage and association with the disease locus using the TDT. The PREFIX= option in the PROC FAMILY statement specifies that the two markers be named Marker1 and Marker2 in the output data set. The CONTCORR option indicates that the continuity correction of 0.5 should be used in calculating the chi-square statistic. The AFFECTED= option of the

TRAIT statement specifies which value of the variable disease should be considered "affected." Note that the pedigree ID variable is listed in the ID statement; however, it is not necessary for this data set, since all the individual IDs are unique. The same results would be obtained if this variable were omitted.

Here is the output data set that is produced:



```
                              ChiSq      df       Prob
     Obs       Locus           TDT       TDT       TDT

      1       Marker1        1.57143      6       0.9546
      2       Marker2        5.79861      5       0.3263
```

Figure 4.1. Statistics for the TDT

Figure 4.1 displays the statistics for the TDT. Since both markers are multiallelic, a joint test of all alleles at each marker is performed by default. The degrees of freedom (in the dfTDT column) indicate that there are seven alleles at Marker1 and six alleles at Marker2, since df$= k - 1$ where k is the number of marker alleles. The ProbTDT column shows that neither of the markers is significantly linked and associated with the disease locus.

Syntax

The following statements are available in PROC FAMILY.

> **PROC FAMILY** < *options* > **;**
> **BY** *variables* **;**
> **ID** *variables* **;**
> **TRAIT** *variable* </ **AFFECTED=***value*> **;**
> **VAR** *variables* **;**

Items within angle brackets (< >) are optional, and statements following the PROC FAMILY statement can appear in any order. The ID, TRAIT, and VAR statements are required. The syntax of each statement is described in the following section in alphabetical order after the description of the PROC FAMILY statement.

PROC FAMILY Statement

PROC FAMILY < *options* > **;**

You can specify the following options in the PROC FAMILY statement.

COMBINE

specifies that the combined versions of the S-TDT and SDT be performed. Thus, families containing parental genotypes can be analyzed under certain conditions using the TDT, and otherwise the specified sibling test is performed. Note that if TDT is also being performed, the TDT is done independently of any other tests. By default, the combined versions are not used.

CONTCORR
CC

specifies that a continuity correction of 0.5 should be used for the TDT, S-TDT, and RC-TDT tests in their asymptotic normal approximations. By default, no correction is used.

DATA=*SAS-data-set*

names the input SAS data set to be used by PROC FAMILY. The default is to use the most recently created data set.

DELIMITER=*'string'*

indicates the string that is used to separate the two alleles that comprise the genotypes contained in the variables specified in the VAR statement. This option is ignored if GENOCOL is not specified.

GENOCOL

indicates that columns specified in the VAR statement contain genotypes instead of alleles. When this option is specified, there is one column per marker. The genotypes must consist of the two alleles separated by a delimiter.

MULT=JOINT
MULT=MAX

specifies which multiallelic version of the TDT, S-TDT, SDT, and RC-TDT tests should be performed. The joint version of the multiallelic tests combines the analyses for each allele at a marker into one overall test statistic, with degrees of freedom (df) corresponding to the number of alleles at the marker. The max version of the multiallelic tests determines if there is at least one allele with a significant test statistic, using the maximum 1 df statistic over all alleles with a multiple testing adjustment made. By default, the joint version of the multiallelic tests is performed. This option has no effect on biallelic markers.

NDATA=*SAS-data-set*

names the input SAS data set containing names, or identifiers, for the markers used in the output. There must be a NAME variable in this data set, which should contain the same number of rows as there are markers in the input data set specified in the DATA= option. When there are fewer rows than there are markers, markers without a name are named using the PREFIX= option. Likewise, if there is no NDATA= data set specified, the PREFIX= option is used.

OUTSTAT=*SAS-data-set*

names the output SAS data set containing the p-values for the tests specified in the PROC FAMILY statement. When this option is omitted, an output data set is created by default and named according to the DATA*n* convention.

PERMS=*number*

indicates that Monte Carlo estimates of exact p-values for the family-based tests should be calculated using permutation samples instead of the p-values from the asymptotic χ^2 distribution. Large values of *number* (10,000 or more) are usually recommended for accuracy, but long execution times may result, particularly with large data sets. When this option is omitted, no permutations are performed and p-values from the asymptotic χ^2 distribution are reported.

PREFIX=*prefix*

specifies a prefix to use in constructing names for marker variables in all output. For example, if PREFIX=VAR, the names of the variables are VAR1, VAR2, ..., VAR*n*. Note that this option is ignored when the NDATA= option is specified, unless there are fewer names in the NDATA data set than there are markers. If this option is omitted, PREFIX=M is the default when variables contain alleles; if GENOCOL is specified, then the names of the variables specified in the VAR statement are used as the marker names.

RCTDT

requests that the reconstruction-combined TDT (RC-TDT) be performed. If none of the four test options (RCTDT, SDT, STDT, or TDT) are specified, then all four tests are performed by default. Note that error-checking is always performed on families with at least one untyped parent in order to determine whether or not reconstruction of parental genotypes can be attempted.

SDT

requests that the SDT, a nonparametric alternative to the S-TDT, be performed. If none of the four test options (RCTDT, SDT, STDT, or TDT) are specified, then all four tests are performed by default. The COMBINE option can be used with this test to indicate that the combined version of the SDT should be performed.

SEED=*number*

specifies the initial seed for the random number generator used for permuting the data to calculate estimates of exact p-values. This option is ignored if PERMS= is not specified. The value for *number* must be an integer; the computer clock time is used if the option is omitted or an integer less than or equal to 0 is specified. For more details about seed values, refer to *SAS Language Reference: Concepts*.

SHOWALL

indicates that all families and markers should be included in the “Family Summary” table. When this option is omitted, a family is only included in the table for any marker where there is a genotype error according to a Mendelian inconsistency.

STDT

requests that the sibling TDT (S-TDT), which analyzes data from sibships, be performed. If none of the four test options (RCTDT, SDT, STDT, or TDT) are specified, then all four tests are performed by default. The COMBINE option can be used with this test to indicate that the combined version of the S-TDT should be performed.

TDT

requests that the original TDT be performed. If none of the four test options (RCTDT, SDT, STDT, or TDT) are specified, then all four tests are performed by default.

BY Statement

BY *variables* **;**

You can specify a BY statement with PROC FAMILY to obtain separate analyses on observations in groups defined by the BY variables. When a BY statement appears, the procedure expects the input data set to be sorted in order of the BY variables. The *variables* are one or more variables in the input data set.

If your input data set is not sorted in ascending order, use one of the following alternatives:

- Sort the data using the SORT procedure with a similar BY statement.
- Specify the BY statement option NOTSORTED or DESCENDING in the BY statement for the FAMILY procedure. The NOTSORTED option does not mean that the data are unsorted but rather that the data are arranged in groups (according to values of the BY variables) and that these groups are not necessarily in alphabetical or increasing numeric order.
- Create an index on the BY variables using the DATASETS procedure (in Base SAS software).

For more information on the BY statement, refer to the discussion in *SAS Language Reference: Concepts*. For more information on the DATASETS procedure, refer to the discussion in the *SAS Procedures Guide*.

ID Statement

ID *variables* **;**

The ID statement is required and must contain, in the following order, either:

- the pedigree ID, the individual ID, then the two parental ID variables, or
- the individual ID, then the two parental IDs

Thus if only three variables are specified in the ID statement, it is assumed that the pedigree identifier has been omitted. The pedigree ID is not necessary if all the individual identifiers are unique. The individual and two parental ID variables can be either numeric or character, but all three must be of the same type. The pedigree variable, if specified, can be either numeric or character regardless of the type of the other three identifiers.

TRAIT Statement

TRAIT *variable </ AFFECTED=value>* **;**

The TRAIT statement is required and identifies the trait variable. This variable must be binary, but may be either character or numeric. By default, the second value of the TRAIT variable that appears in the input data set is considered to be "affected" for the tests. If you would like to specify a different value for "affected," you may do so by adding the /AFFECTED=value option to the TRAIT statement. For a variable with a numeric format, the number that corresponds to "affected" should be specified (AFFECTED=1); if the variable has a character format, the level that corresponds to "affected" should be specified in quotes (AFFECTED="a").

VAR Statement

VAR *variables* **;**

The VAR statement identifies the variables containing either the marker alleles, or the marker genotypes if GENOCOL is specified. By default, the VAR statement should contain $2m$ variable names, where m is the number of markers in the data set. Note that alleles for the same marker should be listed consecutively. When GENOCOL is specified, there should be one variable per marker.

Details

Statistical Computations

For all tests, it is assumed that the marker has two alleles, M_1 and M_2. Extensions to multiallelic markers are made by performing the tests on each allele in turn, with the current allele being considered to be M_1 and all other alleles considered to be M_2. When the CONTCORR option is specified in the PROC FAMILY statement, the z score statistics of all versions of the TDT, S-TDT, and RC-TDT can be continuity corrected by subtracting 0.5 from the absolute value of the numerator. The two-sided p-value for each z score using the normal distribution is equivalent to using the p-value from the χ^2_1 distribution for the square of the z score, and this chi-square form of the statistic is reported in the output data set.

TDT

The TDT (Spielman, McGinnis, and Ewens 1993) is implemented using a normal approximation. This test includes families where both parents have been genotyped for the marker and at least one is heterozygous. If only one parent has been genotyped, that parent is heterozygous, and the affected child is not homozygous and does not have the same genotype as the typed parent, then the TDT can be applied to this family as well (Curtis and Sham 1995). The TDT tests for equality between the proportion of times a heterozygous parent transmits the M_1 allele to an affected child and the proportion of times a heterozygous parent transmits the M_2 allele to an affected child. The normal approximation to the binomial is used to form the z score statistic

$$Z = \frac{b - \frac{b+c}{2}}{\sqrt{\frac{b+c}{4}}}$$

where b is the number of M_1 alleles in all affected children from heterozygous parents and c is the number of M_2 alleles in affected children from heterozygous parents.

Two extensions to a multiallelic TDT are available. The first, which is performed by default or when MULT=JOINT is specified in the PROC FAMILY statement, combines the TDT for each of k alleles at a marker into one statistic as follows (Spielman and Ewens 1996):

$$T_J = \frac{k-1}{k} \sum_{v=1}^{k} Z_v^2$$

where Z_v is simply the Z defined in the preceding paragraph, with allele M_v treated as M_1 and all other alleles as M_2 for each $v = 1, ..., k$. T_J and the continuity-corrected form T'_J have an asymptotic χ^2_{k-1} distribution, and the corresponding p-value is reported.

Alternatively, if the MULT=MAX option is specified, either z_m or z'_m (when the CONTCORR option is specified) is used, where $z_m = \max_{1 \leq v \leq k} |Z_v|$. The equivalent one degree of freedom chi-square statistic is reported, and a Bonferroni correction is applied to its p-value.

Note: The TDT is a valid test of linkage and association only when the data consist of unrelated nuclear families and each family contains only one affected child. Otherwise, it is a valid test of linkage only.

S-TDT

The z score procedure given by Spielman and Ewens (1998) is used to calculate p-values for the S-TDT. This test can be applied to families where there are at least one affected sibling and one unaffected sibling, and not all siblings have the same genotype. The z score, whose two-sided p-value is approximated using the normal distribution, is calculated as $z = (Y - A)/\sqrt{V}$. Y represents the total observed number of M_1 alleles in the affected siblings. For t total siblings in the family, a affected and u unaffected, and r that are M_1/M_1 and s that are M_1/M_2, summing over families gives

$$A = \sum (2r + s)a/t$$

and

$$V = \sum au[4r(t - r - s) + s(t - s)]/[t^2(t - 1)]$$

as the expected value and variance of Y respectively.

When the COMBINE option is specified in the PROC FAMILY statement, the S-TDT and TDT are combined as follows: the TDT is applied to all alleles within a family that meet the requirements described in the preceding section. The S-TDT is then applied to the remaining alleles within a family that meet its requirements described in the preceding paragraph. Using the notation already given for these tests, the z score for the combined test can then be written as

$$z = \frac{(Y+b) - (A + \frac{b+c}{2})}{\sqrt{V + \frac{b+c}{4}}}$$

For multiallelic markers, the same extensions can be made to the S-TDT and combined S-TDT that were made to the TDT (Monks, Kaplan, and Weir 1998); that is, either a joint test over all alleles (using T_{mcomb}), or the maximum z score of all the alleles with the p-value being Bonferroni-corrected.

Note: The S-TDT is a valid test of linkage and association only when the data consist of unrelated nuclear families and each family contains only one affected and one unaffected sibling. Otherwise, it is a valid test of linkage only.

SDT

The SDT (Horvath and Laird 1998) is a sign test used on discordant sibling pairs. As with the S-TDT, one affected sibling and one unaffected sibling are required to be in each family, but unlike the S-TDT, the SDT remains a valid test of linkage and association when the sibship is larger.

The notation from the S-TDT is used, except now the quantities a, u, r, s, and Y are defined for each sibship/family, so for example, there are a affected siblings in the family and u unaffected siblings in the family. Treating each allele M_v in turn as M_1 and all other alleles as M_2, $v = 1, ..., k$, define for each family in the data the average number of v alleles among affected siblings and unaffected siblings respectively as

$$m_v^a = Y/a$$

$$m_v^u = \left[(2r+s) - Y\right]/u$$

Then $d_v = m_v^a - m_v^u$ for each family, and summing over families gives $S_v = \sum \text{sgn}(d_v)$, where $\text{sgn}(d_v) = 1$ for $d_v > 0$, 0 for $d_v = 0$, and -1 for $d_v < 0$. The joint multiallelic SDT statistic (mSDT) is then defined by Czika and Berry (2002) as $T = \mathbf{S}'\mathbf{W}^{-}\mathbf{S}$ where $\mathbf{S}' = (S_1, ..., S_k)'$ and $W_{vw} = \sum \text{sgn}(d_v)\text{sgn}(d_w)$, $v, w = 1, ..., k$, and $\mathbf{W}^{-}$ is the Moore-Penrose generalized inverse of $\mathbf{W}$. T has an asymptotic $\chi^2_{k'}$ distribution where $k' = \text{rank}(\mathbf{W})$, and this distribution is used to obtain p-values for the SDT (Czika and Berry 2002). When there are only two alleles at the marker, this joint multiallelic version of the SDT reduces to the biallelic version of the SDT.

This sibship test is also combined with the TDT when the COMBINE option in the PROC FAMILY statement is specified, creating a test which can potentially use more of the data (Horvath and Laird 1998; Curtis, Miller, and Sham 1999). In order to maintain the test's validity as a test of association in families with more than one affected and one unaffected sibling, a nonparametric multiallelic TDT is used, which is in the same $\mathbf{S}'\mathbf{W}^{-}\mathbf{S}$ form as the SDT. This test statistic for the joint test also has an asymptotic $\chi^2_{k'}$ distribution (Czika and Berry 2002), and the corresponding p-value is reported.

When the MULT=MAX option is specified in the PROC FAMILY statement, then the SDT chi-square statistic is simply $\max_{1 \le v \le k}(S_v^2 W_{vv}^{-1})$ and has one degree of freedom. This applies to the SDT when used alone or combined with the TDT. As with the other tests, a Bonferroni correction is made to the p-value.

RC-TDT

The RC-TDT (Knapp 1999a) takes the combined S-TDT a step further by reconstructing missing parental genotypes when possible in order to use more families. The RC-TDT can be applied to families with at least one affected child that meet one of the following conditions:

- Both parents are typed with at least one heterozygous for M_1.
- One parent is typed, the other can be reconstructed, and at least one parent is heterozygous for M_1.
- Both parents' genotypes are missing but can be reconstructed, and at least one parent is heterozygous for M_1.
- At least one parental genotype is missing and cannot be reconstructed, but the conditions for the S-TDT are met.
- One parental genotype is missing and cannot be reconstructed, the other parent is heterozygous for M_1, at least one affected child is heterozygous for M_1 and an allele not in the typed parent (Knapp 1999b).

Reconstruction of parental genotypes is only attempted when there are no genotyping errors in the family for the marker being tested. As with the S-TDT, a z score is created using the statistic Y, but Knapp (1999a) calculates a different expected value e and variance v of Y, which takes into account the bias created by the genotype reconstruction, to form the z score over all families:

$$z = (Y - e)/\sqrt{v}$$

For multiallelic markers, the same extensions can be made to the RC-TDT that were made to the TDT and S-TDT; that is, either a joint test over all alleles, or the maximum z score of all the alleles with the p-value being Bonferroni-corrected.

Note: The RC-TDT is a valid test of linkage and association only when the data consist of unrelated nuclear families and each family contains only one affected and one unaffected sibling. Otherwise, it is a valid test of linkage only.

Permutation Tests

By default, p-values from the asymptotic χ^2 distribution with appropriate degrees of freedom are reported for all tests. However, if the PERMS= option is specified in the PROC FAMILY statement, then Monte Carlo estimates of exact p-values are calculated using the permutation procedure for the TDT, S-TDT, SDT, and combined S-TDT and SDT. When the TDT is being performed, including when it is performed in the combined tests, new samples are formed by permuting the alleles that are transmitted to the offspring from the parents and those that are not transmitted (Kaplan, Martin, and Weir 1997). Each affected child in a nuclear family is assigned a genotype comprised of one allele from each parent, with each allele being randomly selected from the pair possessed by an individual parent. When the sibling tests are used and the parental information is ignored, the permutation procedure involves randomly permuting the affection status of siblings within each sibship (Spielman and Ewens 1998; Monks, Kaplan, and Weir 1998). For each test, the corresponding test statistic is calculated for the original sample as well as each of the permuted samples. The approximation to the exact p-value is then calculated as the number of times the test statistic from a permuted sample exceeds the test statistic from the original sample.

Missing Values

An individual's genotype for a marker is considered missing if at least one of the alleles at the marker is missing. Any missing genotypes are excluded from all calculations. However, the individual's nonmissing genotypes at other loci can be used as part of the calculations. If a child has a missing trait value, then that individual is excluded from all calculations. However, missing trait values of individuals used only as parents do not affect the analysis. See the following section for information on missing values in the ID variables.

DATA= Data Set

The DATA= data set has columns representing markers, ID variables, and a trait, and rows representing the individuals. There must be one binary trait variable listed in the TRAIT statement; the three ID variables consisting of the individual's ID and the two parental IDs, all of the same type, must be listed in the ID statement, and optionally the pedigree ID if the individual identifiers are not unique. Note that only individuals with both parents appearing in the data, even if all the parents' genotypes are missing, can be used as affected children or in sib-pairs for analysis. However, if the individual is used only as a parent, then that individual's parents need not appear in the data. Also, if a pedigree ID variable is specified in the ID statement, any individual with a missing value for that variable is excluded from the analysis, as a parent and as a child. There are two columns for each marker, representing the two alleles at that marker carried by the individual. These two columns must be listed consecutively in the VAR statement. These marker variables must all be of the same type, but can be either character or numeric variables.

OUTSTAT= Data Set

The OUTSTAT= data set contains the following variables:

- the BY variables, if any
- Locus
- the chi-square statistics for each test performed: ChiSqTDT, ChiSqSTDT, ChiSqSDT, and ChiSqRCTDT
- the degrees of freedom for each test performed: dfTDT, dfSTDT, dfSDT, and dfRCTDT
- the p-values for each test performed: ProbTDT, ProbSTDT, ProbSDT, and ProbRCTDT

Displayed Output

This section describes the displayed output from PROC FAMILY. See the section "ODS Table Names" on page 75 for details about how this output interfaces with the Output Delivery System.

Family Summary

The "Family Summary" table lists information about the nuclear families, including the pedigree ID (if listed in the ID statement) and the two parental IDs, then the following information for each marker locus:

- number of typed parents
- number of affected children with nonmissing genotypes
- number of unaffected children with nonmissing genotypes
- error code

Note that when SHOWALL is specified in the PROC FAMILY statement, all families and all markers are displayed in the table. Otherwise, only families with a Mendelian genotype error and the marker at which they have the error are included in the table. The error code is an integer that represents a particular type of genotype error that is described in the "Description of Error Codes" table.

Description of Error Codes

The "Description of Error Codes" table provides a description for the error codes listed in the "Family Summary" table. The descriptions of the family genotype errors all refer to Mendelian inconsistencies in the child(ren)'s genotypes with the parental genotypes. Error codes 1 through 5 can occur when neither of the parental genotypes for that marker are available (the sibship is the family unit). Codes 6 and 7 can occur for families with exactly one parent genotyped, and error code 8 can occur in families with both parents genotyped.

ODS Table Names

PROC FAMILY assigns a name to each table it creates, and you must use this name to reference the table when using the Output Delivery System (ODS). These names are listed in the following table.

Table 4.1. ODS Tables Created by the FAMILY Procedure

ODS Table Name	Description	PROC FAMILY Option
FamilySummary	Family summary	default
ErrorCodeDesc	Description of error codes	default

Examples

Example 4.1. Performing Tests with Missing Parental Data

The following data are from GAW9 (Hodge 1995) and contain 20 nuclear families that are genotyped at two markers. The data have been modified so that each mother's genotype is missing.

```
data gaw;
   input ped id f_id m_id sex disease m11 m12 m21 m22;
   datalines;
 1    1    0    0 1 1  7  8  7  2
 1    2    0    0 2 1  .  .  .  .
 1  401    1    2 1 1  7  2  7  6
 1  402    1    2 1 1  8  2  7  6
 1  403    1    2 1 1  7  2  2  7
 1  404    1    2 2 2  8  2  7  7
 2    3    0    0 1 1  4  4  1  3
 2    4    0    0 2 1  .  .  .  .
 2  405    3    4 2 1  4  6  1  7
 2  406    3    4 2 2  4  4  3  7
 3    5    0    0 1 1  6  7  7  2
 3    6    0    0 2 1  .  .  .  .
 3  407    5    6 2 2  7  4  7  7
 4    7    0    0 1 1  1  8  7  3
 4    8    0    0 2 1  .  .  .  .
 4  408    7    8 2 2  8  4  7  3
 4  409    7    8 1 1  1  2  3  3
 4  410    7    8 2 1  8  2  7  3
 4  411    7    8 1 1  8  2  7  5
 5    9    0    0 1 1  7  1  6  2
 5   10    0    0 2 1  .  .  .  .
 5  412    9   10 2 2  7  6  6  2
 5  413    9   10 1 1  1  6  6  2
 6   11    0    0 1 1  8  4  2  3
 6   12    0    0 2 1  .  .  .  .
 6  414   11   12 1 2  8  1  2  7
 6  415   11   12 1 1  8  6  3  7
 7   13    0    0 1 1  4  6  2  2
 7   14    0    0 2 1  .  .  .  .
 7  416   13   14 1 1  4  5  2  7
 7  417   13   14 2 2  6  4  2  7
 7  418   13   14 2 1  6  5  2  6
 7  419   13   14 1 1  6  5  2  6
 8   15    0    0 1 1  6  8  2  7
 8   16    0    0 2 1  .  .  .  .
 8  420   15   16 2 1  6  2  7  7
 8  421   15   16 2 1  8  6  2  7
 8  422   15   16 2 2  6  6  7  7
 8  423   15   16 2 1  6  6  7  7
 9   17    0    0 1 2  4  7  2  7
 9   18    0    0 2 1  .  .  .  .
 9  424   17   18 2 2  4  5  7  2
 9  425   17   18 2 1  7  4  2  7
 9  426   17   18 1 1  4  5  2  2
10   19    0    0 1 1  6  4  2  7
10   20    0    0 2 1  .  .  .  .
10  427   19   20 2 2  4  4  7  2
11   21    0    0 1 1  4  7  7  7
11   22    0    0 2 1  .  .  .  .
11  428   21   22 1 1  7  6  7  2
11  429   21   22 2 2  7  4  7  2
11  430   21   22 2 1  7  6  7  3
12   23    0    0 1 1  7  6  7  5
12   24    0    0 2 1  .  .  .  .
12  431   23   24 1 2  6  4  7  7
13   25    0    0 1 1  4  1  2  8
13   26    0    0 2 1  .  .  .  .
13  432   25   26 1 1  4  8  2  6
13  433   25   26 1 2  1  8  8  6
13  434   25   26 1 1  1  4  2  6
14   27    0    0 1 1  7  6  3  2
14   28    0    0 2 1  .  .  .  .
14  435   27   28 1 1  6  2  3  3
14  436   27   28 1 1  7  4  3  7
14  437   27   28 1 1  6  2  2  7
14  438   27   28 1 1  7  4  2  7
14  439   27   28 2 2  6  2  2  7
14  440   27   28 1 1  6  4  3  7
15   29    0    0 1 1  2  4  7  4
15   30    0    0 2 1  .  .  .  .
15  441   29   30 1 1  4  2  7  7
15  442   29   30 2 2  4  8  4  7
15  443   29   30 2 1  4  2  7  5
15  444   29   30 2 1  4  2  7  5
15  445   29   30 1 1  2  8  7  5
;
```

Since there are missing parental data, the original TDT may not be the best test to perform on this data set. The following analysis uses the S-TDT, SDT, and RC-TDT to test markers for linkage with the disease locus.

```
proc family data=gaw prefix=Marker sdt stdt rctdt;
   id id f_id m_id;
   var m11 m12 m21 m22;
   trait disease / affected=2;
run;

proc print;
run;
```

The output data set, which is created by default, is displayed in Output 4.1.1.

Output 4.1.1. Output Data Set from PROC FAMILY

```
               ChiSq    ChiSq    ChiSq    df   df    df     Prob   Prob  Prob
Obs   Locus     STDT     SDT     RCTDT   STDT  SDT  RCTDT   STDT    SDT  RCTDT

 1   Marker1   5.6179   4.0083   4.7398    6    7     6    0.467  0.779  0.578
 2   Marker2  12.6191  10.7500  11.9388    7    8     7    0.082  0.216  0.103
```

Since only one parent is missing genotype information in each nuclear family, the TDT might be applicable to some of the families. The COMBINE option can be specified to use the TDT in the appropriate families, and the S-TDT or SDT for all other families. This option does not apply to the RC-TDT, so that test is omitted from this analysis.

```
proc family data=gaw prefix=Marker tdt sdt stdt combine;
   id id f_id m_id;
   var m11 m12 m21 m22;
   trait disease / affected=2;
run;

proc print;
run;
```

The output data set is displayed in Output 4.1.2.

Output 4.1.2. Output Data Set from PROC FAMILY Using COMBINE Option

```
                ChiSq    ChiSq    ChiSq    df   df    df    Prob   Prob   Prob
Obs   Locus      TDT     STDT      SDT    TDT  STDT  SDT    TDT    STDT    SDT

 1   Marker1  4.44444   6.3692   4.2380   5     6     7   0.487  0.383  0.752
 2   Marker2  2.00000  11.6489  10.7500   3     7     8   0.572  0.113  0.216
```

Note that the test statistics for the TDT and the S-TDT and SDT are not the same; this implies that not all families meet the requirements for the TDT. In this case, the

S-TDT, SDT, and RC-TDT use more of the data than the TDT alone. However, since there is only one affected child in each nuclear family, the TDT is a valid test of association; since there is at least one occasion when there is more than one unaffected child in a nuclear family, the S-TDT and RC-TDT are not valid for testing for association of the marker with the disease locus (the SDT is always a valid test of association when the data consist of unrelated nuclear families). Both of these considerations, the amount of information that can be used and the validity for testing association, should be taken into account when deciding which test(s) to perform.

Another type of analysis can be performed using the MULT=MAX option in the PROC FAMILY statement. This option indicates that instead of doing a joint test over all the alleles at each marker, perform a test to see if any of the alleles at a marker are significantly linked with the disease locus. This analysis is invoked with the following code, using only the SDT and RC-TDT:

```
proc family data=gaw prefix=Marker sdt rctdt combine mult=max;
   id id f_id m_id;
   var m11 m12 m21 m22;
   trait disease / affected=2;
run;

proc print;
run;
```

The output data set produced by this code is displayed in Output 4.1.3.

Output 4.1.3. Output Data Set from PROC FAMILY Using MULT=MAX Option

```
                      ChiSq      ChiSq       df      df         Prob       Prob
Obs      Locus         SDT       RCTDT      SDT    RCTDT         SDT      RCTDT

 1      Marker1      2.66667    2.90050      1       1        0.7173     0.6199
 2      Marker2      3.57143    3.86422      1       1        0.4703     0.3946
```

The chi-square statistics for the tests always have one degree of freedom when the MULT=MAX option is used. Note, however, that the p-values are not the corresponding right-tailed probabilities for a χ^2_1 statistic; this is because the p-values are Bonferroni-corrected in order to account for taking the maximum of several chi-square statistics.

Example 4.2. Checking for Genotyping Errors

This example demonstrates the different kinds of family genotype errors (that is, Mendelian inconsistencies within a nuclear family) that can be detected by PROC FAMILY, and the output that displays this information. Here is a sample data set that contains genotype errors:

```
data ped_samp;
   input id p1 p2 a1 a2 dis;
   datalines;
  1   0   0 1 1 0
  2   0   0 2 3 0
  3   1   2 1 2 0
  4   1   2 4 5 1
101   0   0 . . 0
102   0   0 2 3 0
103 101 102 4 5 1
104 101 102 2 4 1
201   0   0 . . 0
202   0   0 1 4 0
203 201 202 1 5 1
204 201 202 1 6 0
205 201 202 1 7 1
301   0   0 . . 0
302   0   0 . . 0
303 301 302 1 2 1
304 301 302 1 3 0
305 301 302 1 4 0
401   0   0 . . 0
402   0   0 . . 0
403 401 402 1 1 1
404 401 402 2 2 1
405 401 402 3 3 0
501   0   0 . . 0
502   0   0 . . 0
503 501 502 1 1 0
504 501 502 2 2 0
505 501 502 1 3 1
601   0   0 . . 0
602   0   0 . . 0
603 601 602 1 1 1
604 601 602 1 4 0
605 601 602 2 3 0
701   0   0 . . 0
702   0   0 . . 0
703 701 702 1 2 0
704 701 702 2 3 1
705 701 702 1 4 0
707 701 702 2 5 1
801   0   0 1 3 0
802   0   0 . . 0
804 801 802 1 4 1
805 801 802 3 2 1
;
```

In addition to the usual output data set that is created, the SHOWALL option requests that all families be included in the "Family Summary" table. Since there are families with genotype errors, this table would have been created by default, but only the families in error would be displayed in it.

```
proc family data=ped_samp showall;
   id id p1 p2;
   trait dis;
   var a1 a2;
run;

proc print;
run;
```

The “Family Summary” table shown in Output 4.2.1 includes an error code, which is explained in the “Description of Error Codes” table in Output 4.2.2. The statistics shown in Output 4.2.3 are based only on the last family since all the other families have some sort of genotype error and thus are excluded from the analyses.

Output 4.2.1. Summary of Family/Marker Information

```
                          The FAMILY Procedure

                            Family Summary

                                    Number      Number        Number
                                        of          of            of
                                     Typed    Affected    Unaffected     Error
Parent1      Parent2      Locus    Parents    Children      Children      Code

1            2            M1             2           1             1         8
101          102          M1             1           2             0         6
201          202          M1             1           2             1         7
301          302          M1             0           1             2         5
401          402          M1             0           2             1         4
501          502          M1             0           1             2         3
601          602          M1             0           1             2         2
701          702          M1             0           2             2         1
801          802          M1             1           2             0         0
```

Output 4.2.2. Description of Error Codes

```
                       Description of Error Codes

     Code    Description

        0    No errors
        1    More than 4 alleles
        2    1 homozygous genotype and more than 3 alleles
        3    2 homozygous genotypes and more than 2 alleles
        4    More than 2 homozygous genotypes
        5    An allele occurs in more than 2 heterozygous genotypes
        6    At least one genotype does not contain a parental allele
        7    More than 2 alleles from missing parent
        8    At least one genotype incompatible with parental genotypes
```

Output 4.2.3. Output Data Set from PROC FAMILY

```
                          Chi         Chi
                Chi        Sq   Chi    Sq   df   df   df    df  Prob Prob Prob  Prob
Obs Locus SqTDT  STDT SqSDT RCTDT TDT STDT SDT RCTDT  TDT STDT  SDT RCTDT

 1   M1     0     0     0     0    1    0   0    1     1    .    .    1
```

References

Curtis, D., Miller, M.B., and Sham, P.C. (1999), "Combining the Sibling Disequilibrium Test and Transmission/Disequilibrium Test for Multiallelic Markers," *American Journal of Human Genetics,* 64, 1785–1786.

Curtis, D. and Sham, P.C. (1995), "A Note on the Application of the Transmission Disequilibrium Test When a Parent is Missing," *American Journal of Human Genetics,* 56, 811–812.

Czika, W. and Berry, J.J. (2002), "Using All Alleles in the Multiallelic Versions of the SDT and Combined SDT/TDT," *American Journal of Human Genetics,* 71, 1235–1236.

Hodge, S.E. (1995), "An Oligogenic Disease Displaying Weak Marker Associations: A Summary of Contributions to Problem 1 of GAW9," *Genetic Epidemiology,* 12, 545–554.

Horvath, S. and Laird, N.M. (1998), "A Discordant-Sibship Test for Disequilibrium and Linkage: No Need for Parental Data," *American Journal of Human Genetics,* 63, 1886–1897.

Kaplan, N.L., Martin, E.R., and Weir, B.S. (1997), "Power Studies for the Transmission/Disequilibrium Tests with Multiple Alleles," *American Journal of Human Genetics,* 60, 691–702.

Knapp, M. (1999a), "The Transmission/Disequilibrium Test and Parental-Genotype Reconstruction: The Reconstruction-Combined Transmission/Disequilibrium Test," *American Journal of Human Genetics,* 64, 861–870.

Knapp, M. (1999b), "Using Exact P Values to Compare the Power between the Reconstruction-Combined Transmission/Disequilibrium Test and the Sib Transmission/Disequilibrium Test," *American Journal of Human Genetics,* 65, 1208–1210.

Monks, S.A., Kaplan, N.L., and Weir, B.S. (1998), "A Comparative Study of Sibship Tests of Linkage and/or Association," *American Journal of Human Genetics,* 63, 1507–1516.

Spielman, R.S. and Ewens, W.J. (1996), "The TDT and Other Family-based Tests for Linkage Disequilibrium and Association," *American Journal of Human Genetics,* 59, 983–989.

Spielman, R.S. and Ewens, W.J. (1998), “A Sibship Test for Linkage in the Presence of Association: The Sib Transmission/Disequilibrium Test,” *American Journal of Human Genetics,* 62, 450–458.

Spielman, R.S., McGinnis, R.E., and Ewens, W.J. (1993), “Transmission Test for Linkage Disequilibrium: The Insulin Gene Region and Insulin-dependent Diabetes Mellitus (IDDM),” *American Journal of Human Genetics,* 52, 506–516.

Chapter 5
The HAPLOTYPE Procedure

Chapter Contents

Chapter 5
The HAPLOTYPE Procedure

Overview

A *haplotype* is a combination of alleles at multiple loci on a single chromosome. A pair of haplotypes constitutes the multilocus genotype. Haplotype information has to be inferred as data are usually collected at the genotypic, not haplotype pair, level. For homozygous markers, there is no problem. If one locus has alleles A and a, and a second locus has alleles B and b, the observed genotype $AABB$ must contain two haplotypes of type AB; genotype $AaBB$ must contain haplotypes AB and aB, and so on. Haplotypes and their frequencies can be obtained directly. When both loci are heterozygous, however, there is ambiguity; a variety of combinations of haplotypes can generate the genotype, and it is not possible to determine directly which two haplotypes constitute any individual genotype. For example, the genotype $AaBb$ may be of type AB/ab with haplotypes AB and ab, or of type Ab/aB with haplotypes Ab and aB. The HAPLOTYPE procedure uses the expectation-maximization (EM) algorithm to generate maximum likelihood estimates of haplotype frequencies given a multilocus sample of genetic marker genotypes under the assumption of Hardy-Weinberg equilibrium (HWE). These estimates can then in turn be used to assign the probability that each individual possesses a particular haplotype pair.

Estimation of haplotype frequencies is important for several applications in genetic data analysis. One application is determining whether there is linkage disequilibrium (LD), or association, between loci. PROC HAPLOTYPE performs a likelihood ratio test to test the hypothesis of no LD between marker loci. Another application is association testing of disease susceptibility. Since sites that affect disease status are embedded in haplotypes, it has been postulated that the power of case-control studies might be increased by testing for haplotype rather than allele or genotype associations. One reason is that haplotypes might include two or more causative sites whose combined effect is measurable, particularly if they show synergistic interaction. Another is that fewer tests need be performed, although if there are a large number of haplotypes, this advantage is offset by the increased degrees of freedom of each test. PROC HAPLOTYPE can use case-control data to calculate test statistics for the hypothesis of no association between alleles comprising the haplotypes and disease status; such tests are carried out over all haplotypes at the loci specified, or for individual haplotypes.

Getting Started

Example

Assume you have a random sample with 25 individuals genotyped at four markers. You want to infer the gametic phases of the genotypes and estimate their frequencies. There are eight columns of data, with the first two columns containing the pair of alleles at the first marker, and the next two columns containing the pair of alleles for the second marker, and so on. Each row represents an individual. The data can be read into a SAS data set as follows:

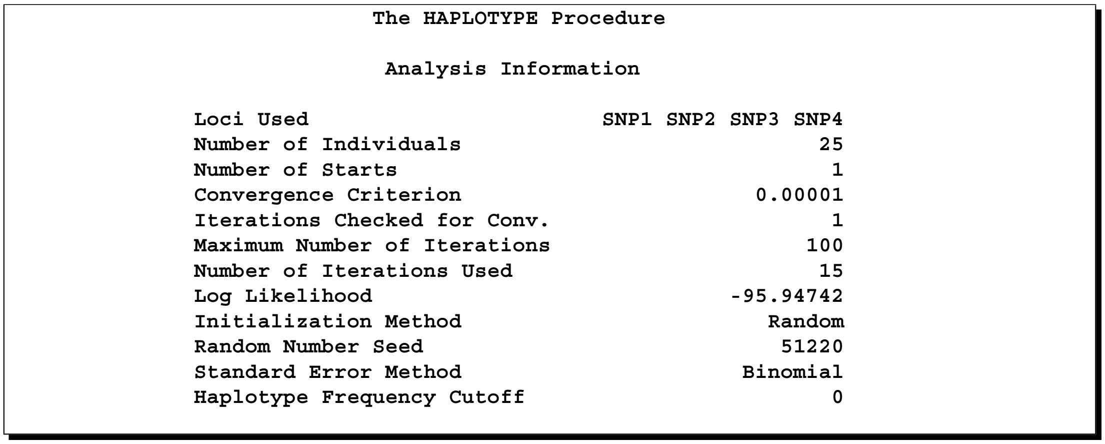
```
data markers;
   input (m1-m8) ($);
   datalines;
B  B  A  B  B  B  A  A
A  A  B  B  A  B  A  B
B  B  A  A  B  B  B  B
A  B  A  B  A  B  A  B
A  A  A  B  A  B  B  B
B  B  A  A  A  B  A  B
A  B  B  B  A  B  A  A
A  B  A  A  A  A  A  A
B  B  A  A  A  A  A  B
A  B  A  B  A  B  B  B
A  B  A  B  A  B  A  A
B  B  A  B  A  B  A  A
A  B  A  A  A  B  A  B
A  B  B  B  B  B  A  B
A  A  A  B  A  A  A  B
B  B  A  B  A  B  A  B
A  B  B  B  A  A  A  B
B  B  B  B  A  A  A  A
A  B  A  A  A  B  A  A
A  B  A  A  A  B  A  B
B  B  A  A  A  A  A  B
A  A  A  B  A  A  A  B
A  B  A  A  A  A  B  B
A  A  A  A  A  A  A  A
A  B  B  B  A  A  A  A
;
```

You can now use PROC HAPLOTYPE to infer the possible haplotypes and estimate the four-locus haplotype frequencies in this sample. The following statements will perform these calculations:

```
proc haplotype data=markers out=hapout init=random prefix=SNP;
   var m1-m8;
run;

proc print data=hapout noobs round;
run;
```

This analysis uses the EM algorithm to estimate the haplotype frequencies from the sample. The standard errors and a confidence interval are estimated, by default, under a binomial assumption for each haplotype frequency estimate. A more precise estimate of the standard error can be obtained through the jackknife process by specifying the option SE=JACKKNIFE in the PROC HAPLOTYPE statement, but it takes considerably more computations (see the "Methods for Estimating Standard Error" section on page 97 for more information). The option INIT=RANDOM indicates that initial haplotype frequencies are randomly generated, using a random seed created by the system clock since the SEED= option is omitted. The default confidence level 0.95 is used since the ALPHA= option of the PROC HAPLOTYPE statement was omitted. Also by default, the convergence criterion of 0.00001 must be satisfied for one iteration, and the maximum number of iterations is set to 100. The PREFIX= option requests that the four markers, indicated by the eight allele variables in the VAR statement, be named SNP1-SNP4.

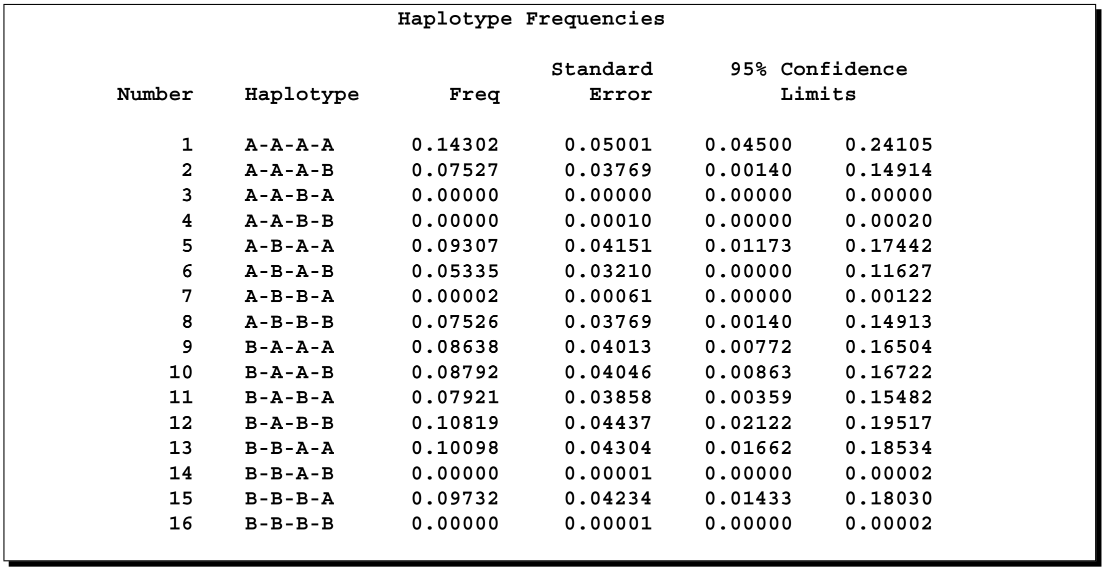
The results from the procedure are as follows.



```
                      The HAPLOTYPE Procedure

                        Analysis Information

          Loci Used                      SNP1 SNP2 SNP3 SNP4
          Number of Individuals                           25
          Number of Starts                                 1
          Convergence Criterion                      0.00001
          Iterations Checked for Conv.                     1
          Maximum Number of Iterations                   100
          Number of Iterations Used                       15
          Log Likelihood                           -95.94742
          Initialization Method                       Random
          Random Number Seed                           51220
          Standard Error Method                     Binomial
          Haplotype Frequency Cutoff                       0
```

Figure 5.1. Analysis Information for the HAPLOTYPE Procedure

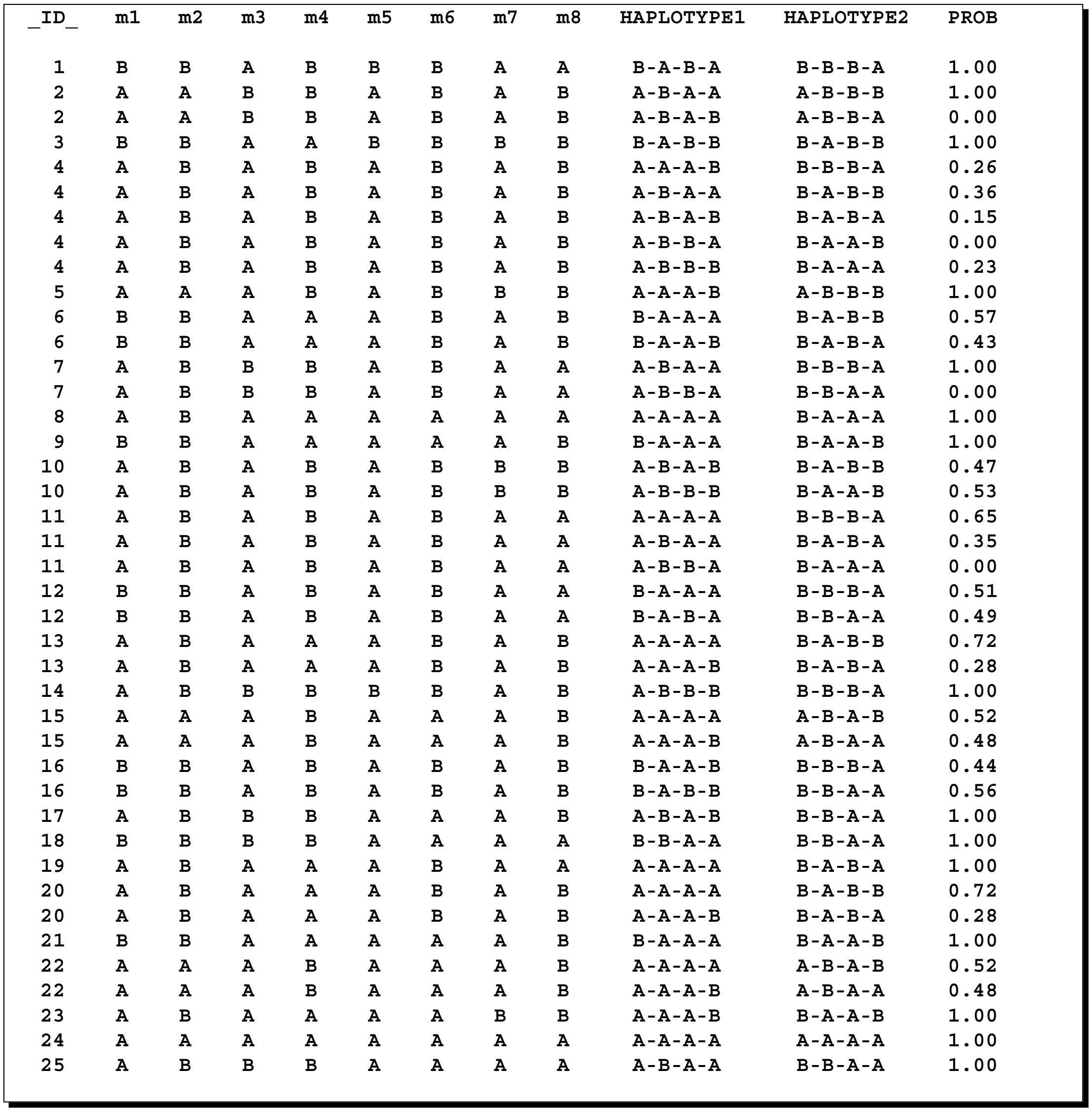
Figure 5.1 displays information on several of the settings used to perform the HAPLOTYPE procedure as well as information on the EM algorithm. Note that you can obtain from this table the random seed that was generated by the system clock if you need to replicate this analysis.



```
                        Haplotype Frequencies

                                          Standard      95% Confidence
      Number     Haplotype        Freq       Error           Limits

           1     A-A-A-A       0.14302     0.05001     0.04500    0.24105
           2     A-A-A-B       0.07527     0.03769     0.00140    0.14914
           3     A-A-B-A       0.00000     0.00000     0.00000    0.00000
           4     A-A-B-B       0.00000     0.00010     0.00000    0.00020
           5     A-B-A-A       0.09307     0.04151     0.01173    0.17442
           6     A-B-A-B       0.05335     0.03210     0.00000    0.11627
           7     A-B-B-A       0.00002     0.00061     0.00000    0.00122
           8     A-B-B-B       0.07526     0.03769     0.00140    0.14913
           9     B-A-A-A       0.08638     0.04013     0.00772    0.16504
          10     B-A-A-B       0.08792     0.04046     0.00863    0.16722
          11     B-A-B-A       0.07921     0.03858     0.00359    0.15482
          12     B-A-B-B       0.10819     0.04437     0.02122    0.19517
          13     B-B-A-A       0.10098     0.04304     0.01662    0.18534
          14     B-B-A-B       0.00000     0.00001     0.00000    0.00002
          15     B-B-B-A       0.09732     0.04234     0.01433    0.18030
          16     B-B-B-B       0.00000     0.00001     0.00000    0.00002
```

Figure 5.2. Haplotype Frequencies from the HAPLOTYPE Procedure

Figure 5.2 displays the possible haplotypes in the sample and their estimated frequencies with standard errors and the lower and upper limits of the 95% confidence interval.



```
 _ID_    m1    m2    m3    m4    m5    m6    m7    m8    HAPLOTYPE1    HAPLOTYPE2    PROB

   1     B     B     A     B     B     B     A     A      B-A-B-A       B-B-B-A      1.00
   2     A     A     B     B     A     B     A     B      A-B-A-A       A-B-B-B      1.00
   2     A     A     B     B     A     B     A     B      A-B-A-B       A-B-B-A      0.00
   3     B     B     A     A     B     B     B     B      B-A-B-B       B-A-B-B      1.00
   4     A     B     A     B     A     B     A     B      A-A-A-B       B-B-B-A      0.26
   4     A     B     A     B     A     B     A     B      A-B-A-A       B-A-B-B      0.36
   4     A     B     A     B     A     B     A     B      A-B-A-B       B-A-B-A      0.15
   4     A     B     A     B     A     B     A     B      A-B-B-A       B-A-A-B      0.00
   4     A     B     A     B     A     B     A     B      A-B-B-B       B-A-A-A      0.23
   5     A     A     A     B     A     B     B     B      A-A-A-B       A-B-B-B      1.00
   6     B     B     A     A     A     B     A     B      B-A-A-A       B-A-B-B      0.57
   6     B     B     A     A     A     B     A     B      B-A-A-B       B-A-B-A      0.43
   7     A     B     B     B     A     B     A     A      A-B-A-A       B-B-B-A      1.00
   7     A     B     B     B     A     B     A     A      A-B-B-A       B-B-A-A      0.00
   8     A     B     A     A     A     A     A     A      A-A-A-A       B-A-A-A      1.00
   9     B     B     A     A     A     A     A     B      B-A-A-A       B-A-A-B      1.00
  10     A     B     A     B     A     B     B     B      A-B-A-B       B-A-B-B      0.47
  10     A     B     A     B     A     B     B     B      A-B-B-B       B-A-A-B      0.53
  11     A     B     A     B     A     B     A     A      A-A-A-A       B-B-B-A      0.65
  11     A     B     A     B     A     B     A     A      A-B-A-A       B-A-B-A      0.35
  11     A     B     A     B     A     B     A     A      A-B-B-A       B-A-A-A      0.00
  12     B     B     A     B     A     B     A     A      B-A-A-A       B-B-B-A      0.51
  12     B     B     A     B     A     B     A     A      B-A-B-A       B-B-A-A      0.49
  13     A     B     A     A     A     B     A     B      A-A-A-A       B-A-B-B      0.72
  13     A     B     A     A     A     B     A     B      A-A-A-B       B-A-B-A      0.28
  14     A     B     B     B     B     B     A     B      A-B-B-B       B-B-B-A      1.00
  15     A     A     A     B     A     A     A     B      A-A-A-A       A-B-A-B      0.52
  15     A     A     A     B     A     A     A     B      A-A-A-B       A-B-A-A      0.48
  16     B     B     A     B     A     B     A     B      B-A-A-B       B-B-B-A      0.44
  16     B     B     A     B     A     B     A     B      B-A-B-B       B-B-A-A      0.56
  17     A     B     B     B     A     A     A     B      A-B-A-B       B-B-A-A      1.00
  18     B     B     B     B     A     A     A     A      B-B-A-A       B-B-A-A      1.00
  19     A     B     A     A     A     B     A     A      A-A-A-A       B-A-B-A      1.00
  20     A     B     A     A     A     B     A     B      A-A-A-A       B-A-B-B      0.72
  20     A     B     A     A     A     B     A     B      A-A-A-B       B-A-B-A      0.28
  21     B     B     A     A     A     A     A     B      B-A-A-A       B-A-A-B      1.00
  22     A     A     A     B     A     A     A     B      A-A-A-A       A-B-A-B      0.52
  22     A     A     A     B     A     A     A     B      A-A-A-B       A-B-A-A      0.48
  23     A     B     A     A     A     A     B     B      A-A-A-B       B-A-A-B      1.00
  24     A     A     A     A     A     A     A     A      A-A-A-A       A-A-A-A      1.00
  25     A     B     B     B     A     A     A     A      A-B-A-A       B-B-A-A      1.00
```

Figure 5.3. Output Data Set from the HAPLOTYPE Procedure

Figure 5.3 displays each individual's genotype with each of the possible haplotype pairs that can comprise the genotype, and the probability the genotype can be resolved into each of the possible haplotype pairs.

Syntax

The following statements are available in PROC HAPLOTYPE.

> **PROC HAPLOTYPE** < *options* > ;
> **BY** *variables* ;
> **ID** *variables* ;
> **TRAIT** *variable* ;
> **VAR** *variables* ;

Items within angle brackets (< >) are optional, and statements following the PROC HAPLOTYPE statement can appear in any order. Only the VAR statement is required. The syntax for each statement is described in the following section in alphabetical order after the description of the PROC HAPLOTYPE statement.

PROC HAPLOTYPE Statement

> **PROC HAPLOTYPE** < *options* > ;

You can specify the following options in the PROC HAPLOTYPE statement.

ALPHA=*number*

specifies that a confidence level of $100(1-\textit{number})\%$ is to be used in forming the confidence intervals for estimates of haplotype frequencies. The value of *number* must be between 0 and 1, inclusive, and 0.05 is used as the default value if it is not specified.

CONV=*number*

specifies the convergence criterion for iterations of the EM algorithm, where $0 < \textit{number} \leq 1$. The iteration process is stopped when the ratio of the change in the log likelihoods to the former log likelihood is less than or equal to *number* for the number of consecutive iterations specified in the NLAG= option (or 1 by default), or after the number of iterations specified in the MAXITER= option has been performed. The default value is 0.00001.

CUTOFF=*number*

specifies a lower bound on a haplotype's estimated frequency in order for that haplotype to be included in the "Haplotype Frequencies" table. The value of *number* must be between 0 and 1, inclusive. By default, all possible haplotypes from the sample are included in the table.

DATA=*SAS-data-set*

names the input SAS data set to be used by PROC HAPLOTYPE. The default is to use the most recently created data set.

DELIMITER=*'string'*

indicates the string that is used to separate the two alleles that comprise the genotypes contained in the variables specified in the VAR statement. This option is ignored if GENOCOL is not specified.

EST=EM
EST=STEPEM

indicates the method to be used for estimating haplotype frequencies. By default or when EST=EM is specified, the EM algorithm is used. When EST=STEPEM, the stepwise EM algorithm is used to calculate estimates of haplotype frequencies.

GENOCOL

indicates that columns specified in the VAR statement contain genotypes instead of alleles. When this option is specified, there is one column per marker. The genotypes must consist of the two alleles separated by a delimiter.

INDIVIDUAL=*variable* *Experimental*
INDIV=*variable*

specifies the individual ID variable when using the experimental TALL option. This variable may be character or numeric.

INIT=LINKEQ
INIT=RANDOM
INIT=UNIFORM

indicates the method of initializing haplotype frequencies to be used in the EM algorithm. INIT=LINKEQ initializes haplotype frequencies assuming linkage equilibrium by calculating the product of the frequencies of the alleles that comprise the haplotype. INIT=RANDOM initializes haplotype frequencies with random values from a Uniform(0,1) distribution, and INIT=UNIFORM assigns equal frequency to all haplotypes. By default, INIT=LINKEQ.

ITPRINT

requests that the “Iteration History” table be displayed. This option is ignored if the NOPRINT option is specified.

LD

requests that haplotype frequencies be calculated under the assumption of no LD, in addition to being calculated using the EM algorithm. When this option is specified, the “Test for Allelic Associations” table is displayed, which contains statistics for the likelihood ratio test for allelic associations. This option is ignored if the NOPRINT option is specified.

MARKER=*variable* *Experimental*

specifies the marker ID variable when using the experimental TALL option. This variable contains the names of the markers that are used in all output and may be character or numeric.

MAXITER=*number*

specifies the maximum number of iterations to be used in the EM algorithm. The number must be a nonnegative integer. Iterations are carried out until convergence is reached according to the convergence criterion, or *number* iterations have been performed. The default is MAXITER=100.

NDATA=*SAS-data-set*

names the input SAS data set containing names, or identifiers, for the markers used in the output. There must be a NAME variable in this data set, which should contain the same number of rows as there are markers in the input data set specified in the DATA= option. When there are fewer rows than there are markers, markers without a name are named using the PREFIX= option. Likewise, if there is no NDATA= data set specified, the PREFIX= option is used. Note that this data set is ignored if the experimental TALL option is specified in the PROC HAPLOTYPE statement. In that case, the marker variable names are taken from the marker ID variable specified in the MARKER= option.

NLAG=*number*

specifies the number of consecutive iterations that must meet the convergence criterion specified in the CONV= option (0.00001 by default) for the iteration process of the EM algorithm to stop. The number must be a positive integer. If this option is omitted, one iteration must satisfy the convergence criterion by default.

NOPRINT

suppresses the display of the "Analysis Information," "Iteration History," "Haplotype Frequencies," and "Test for Allelic Associations" tables. Either the OUT= option, the TRAIT statement, or both must be used with the NOPRINT option.

NSTART=*number*

specifies the number of different starts used for the EM algorithm. When this option is specified, PROC HAPLOTYPE starts the iterations with different random initial values $number-2$ times as well as once with uniform frequencies for all the haplotypes and once using haplotype frequencies assuming linkage equilibrium (independence). Results on the analysis using the initial values that produce the best log likelihood are then reported. The number must be a positive integer. If this option is omitted or NSTART=1, only one start with initial frequencies generated according to the INIT= option is used.

OUT=*SAS-data-set*

names the output SAS data set containing the probabilities of each genotype being resolved into all of the possible haplotype pairs.

OUTCUT=*number*

specifies a lower bound on a haplotype pair's estimated probability given the individual's genotype in order for that haplotype pair to be included in the OUT= data set. The value of *number* must be between 0 and 1, inclusive. By default, $number = 0.00001$. In order to be able to view all possible haplotype pairs for an individual's genotype, OUTCUT=0 can be specified.

OUTID

indicates that the variable _ID_ created by PROC HAPLOTYPE should be included in the OUT= data set in addition to the variable(s) listed in the ID statement. When the ID statement is omitted, this variable is automatically included.

PREFIX=*prefix*

specifies a prefix to use in constructing names for marker variables in all output. For example, if PREFIX=VAR, the names of the variables are VAR1, VAR2, ..., VAR*n*. Note that this option is ignored when the NDATA= option is specified, unless there are fewer names in the NDATA data set than there are markers; it is also ignored if the experimental TALL option is specified, in which case the marker variable names are taken from the marker ID variable specified in the MARKER= option. Otherwise, if this option is omitted, PREFIX=M is the default when variables contain alleles; if GENOCOL is specified, then the names of the variables specified in the VAR statement are used as the marker names.

SE=BINOMIAL
SE=JACKKNIFE

specifies the standard error estimation method. There are two methods available: the BINOMIAL option, which gives a standard error estimator from a binomial distribution and is the default method, and the JACKKNIFE option, which requests that the jackknife procedure be used to estimate the standard error.

SEED=*number*

specifies the initial seed for the random number generator used for creating the initial haplotype frequencies when INIT=RANDOM and/or to permute the data when the PERMUTATION= option of the TRAIT statement is specified. The value for *number* must be an integer; the computer clock time is used if the option is omitted or an integer less than or equal to 0 is specified. For more details about seed values, refer to *SAS Language Reference: Concepts*.

STEPTRIM=*number*

indicates the cutoff to be used for the stepwise EM algorithm when trimming the haplotype table, where $0 < number < 1$. This option is only implemented when EST=STEPEM is specified. By default, this number is set to $\min(0.001, 1/(2n))$ where n is the number of individuals in the data set.

TALL *Experimental*

indicates that the input data set is of an alternative format. This format contains the following columns: two containing marker alleles (or one containing marker genotypes if GENOCOL is specified), one for the marker identifier, and one for the individual identifier. The experimental MARKER= and INDIV= options must also be specified for this option to be in effect. Note that when this option is used, the DATA= data set must first be sorted by any BY variables, then sorted by the marker ID variable, then the individual ID variable.

BY Statement

BY *variables* **;**

You can specify a BY statement with PROC HAPLOTYPE to obtain separate analyses on observations in groups defined by the BY variables. When a BY statement appears, the procedure expects the input data set to be sorted in order of the BY variables. The *variables* are one or more variables in the input data set.

If your input data set is not sorted in ascending order, use one of the following alternatives:

- Sort the data using the SORT procedure with a similar BY statement.
- Specify the BY statement option NOTSORTED or DESCENDING in the BY statement for the HAPLOTYPE procedure. The NOTSORTED option does not mean that the data are unsorted but rather that the data are arranged in groups (according to values of the BY variables) and that these groups are not necessarily in alphabetical or increasing numeric order.
- Create an index on the BY variables using the DATASETS procedure (in base SAS software).

For more information on the BY statement, refer to the discussion in *SAS Language Reference: Concepts*. For more information on the DATASETS procedure, refer to the discussion in the *SAS Procedures Guide*.

ID Statement

ID *variables* **;**

The ID statement identifies the variable(s) from the DATA= data set to be included in the OUT= data set. When this statement is omitted, PROC HAPLOTYPE creates in the OUT= data set the variable _ID_ that contains a unique numeric identifier for each individual.

TRAIT Statement

TRAIT *variable < / options >* **;**

The TRAIT statement identifies the binary variable that indicates which individuals are cases and which are controls, or represents a dichotomous trait. This variable can be character or numeric, but must have only two nonmissing levels. When this statement is used, the "Test for Marker-Trait Association" table is included in the output.

There are two options you can specify in the TRAIT statement:

PERMS= *number*
PERMUTATION=*number*

specifies the number of permutations to be used to calculate the empirical p-value of the haplotype case-control tests. This number must be a positive integer. By default, no permutations are used and the p-value is calculated using the chi-square test statistic. Note that this option can greatly increase the computation time.

TESTALL

specifies that each individual haplotype should be tested for association with the TRAIT variable. When this option is included in the TRAIT statement, the "Tests for Haplotype-Trait Association" table is included in the output.

VAR Statement

VAR *variables* **;**

The VAR statement identifies the variables containing either the marker alleles, or the marker genotypes if GENOCOL is specified. The following number of variables should be specified in this statement for a data set containing m markers according to whether the options GENOCOL and TALL are used:

- When both GENOCOL and TALL are specified, there should be one variable named containing marker genotypes.
- When only TALL is specified, there should be two variables named containing marker alleles.
- When only GENOCOL is specified, there should be m variables named, one for each marker containing marker genotypes.
- When neither option is specified, there should be $2m$ variables named, two for each marker containing marker alleles.

All variables specified must be of the same type, either character or numeric.

Details

Statistical Computations

The EM Algorithm

The EM algorithm (Excoffier and Slatkin 1995; Hawley and Kidd 1995; Long, Williams, and Urbanek 1995) iteratively furnishes the maximum likelihood estimates (MLEs) of m-locus haplotype frequencies, for any integer $m > 1$, when a direct solution for the MLE is not readily feasible. The EM algorithm assumes HWE; it has been argued (Fallin and Schork 2000) that positive increases in the Hardy-Weinberg disequilibrium coefficient (toward excess heterozygosity) may increase the error of the EM estimates, but negative increases (toward excess homozygosity) do not demonstrate a similar increase in the error. The iterations start with assigning initial values to the haplotype frequencies. When the INIT=RANDOM option is included in the PROC HAPLOTYPE statement, uniformly distributed random values are assigned to all haplotype frequencies; when INIT=UNIFORM, each haplotype is given an initial frequency of $1/h$, where h is the number of possible haplotypes in the sample. Otherwise, the product of the frequencies of the alleles that comprise the haplotype is used as the initial frequency for the haplotype. Different starting values can lead to different solutions since a maximum that is found could be a local maximum and not the global maximum. You can try different starting values for the EM algorithm by specifying a number greater than 1 in the NSTART= option to get better estimates. The expectation and maximization steps (E-step and M-step, respectively) are then carried out until the convergence criterion is met or the number of iterations exceeds the number specified in the MAXITER= option of the PROC HAPLOTYPE statement.

For a sample of n individuals, suppose the ith individual has genotype G_i. The probability of this genotype in the population is P_i, so the log likelihood is

$$\log L = \sum_{i=1}^{n} \log P_i$$

which is calculated after each iteration's E-step of the EM algorithm, described in the following paragraphs.

Let h_j be the jth possible haplotype and f_j its frequency in the population. For genotype G_i, the set H_i is the collection of pairs of haplotypes, h_j and its "complement" h_j^{ci}, that constitute that genotype. The haplotype frequencies f_j used in the E-step for iteration 0 of the EM algorithm are given by the initial values; all subsequent iterations use the haplotype frequencies calculated by the M-step of the previous iteration. The E-step sets the genotype frequencies to be products of these frequencies:

$$P_i = \sum_{j \in H_i} f_j f_j^{ci}$$

When G_i has m heterozygous loci, there are 2^{m-1} terms in this sum. The number of times haplotype h_j occurs in the sum is written as m_{ij}, which is 2 if G_i is completely homozygous, and either 1 or 0 otherwise.

The M-step sets new haplotype frequencies from the genotype frequencies:

$$f_j = \frac{1}{2n} \sum_{i=1}^{n} \frac{m_{ij} f_j f_j^{ci}}{P_i}$$

The EM algorithm increases the likelihood after each iteration, and multiple starting points can generally lead to the global maximum.

When the option EST=STEPEM is specified in the PROC HAPLOTYPE statement, a stepwise version of the EM algorithm is performed. A common difficulty in haplotype analysis is that the number of possible haplotypes grows exponentially with the number of loci, as does the computation time, which makes the EM algorithm infeasible for a large number of loci. However, the most common haplotypes can still be estimated by trimming the haplotype table using a given cutoff (Clayton 2002). The two-locus haplotype frequencies are first estimated, and those below the cutoff are discarded from the table. The remaining haplotypes are expanded to the next locus by forming all possible three-locus haplotypes, and the EM algorithm is then invoked for this haplotype table. The trimming and expanding process is performed repeatedly, adding one locus at a time, until all loci are considered.

Once the EM or stepwise EM algorithm has arrived at the MLEs of the haplotype frequencies, each individual i's probability of having a particular haplotype pair (h_j, h_j^{ci}) given the individual's genotype G_i is calculated as

$$\Pr\{h_j, h_j^{ci} | G_i\} = \frac{f_j f_j^{ci}}{P_i}$$

for each $j \in H_i$. These probabilities are displayed in the OUT= data set.

Methods for Estimating Standard Error

Typically, an estimate of the variance of a haplotype frequency is obtained by inverting the estimated information matrix from the distribution of genotype frequencies. However, it often turns out that in a large multilocus system, a certain proportion of haplotypes have ML frequencies equal or close to zero which makes the sample information matrix nearly singular (Excoffier and Slatkin 1995). Therefore, two approximation methods are used to estimate the variances, as proposed by Hawley and Kidd (1995).

The binomial method estimates the standard error by calculating the square root of the binomial variance, as if the haplotype frequencies are obtained by direct counting:

$$\text{Var}_{\text{B}}(f_j) = \frac{f_j(1-f_j)}{2n-1}$$

The jackknife method is a simulation-based method that can be used to estimate the standard errors of haplotype frequencies. Each individual is in turn removed from the sample, and all the haplotype frequencies are recalculated from this “delete-1” sample. Let $T_{n-1,i}$ be the haplotype frequency estimator from the ith “delete-1” sample; then the jackknife variance estimator has the following formula:

$$\text{Var}_{\text{J}}(f_j) = \frac{n-1}{n} \sum_{i=1}^{n} \left(T_{n-1,i} - \frac{1}{n} \sum_{j=1}^{n} T_{n-1,j} \right)^2$$

and the square root of this variance estimate is the estimate of standard error. The jackknife is less dependent on the model assumptions; however, it requires computing the statistic n times.

Confidence intervals with confidence level $1-\alpha$ for the haplotype frequency estimates from the final iteration are then calculated using the following formula:

$$f_j \pm z_{1-\alpha/2} \sqrt{\text{Var}(f_j)}$$

where $z_{1-\alpha/2}$ is the value from the standard normal distribution that has a right-tail probability of $\alpha/2$.

Testing for Allelic Associations

When the LD option is specified in the PROC HAPLOTYPE statement, haplotype frequencies are calculated using the EM algorithm as well as by assuming no allelic associations among loci, that is, no LD. Under the null hypothesis of no LD, haplotype frequencies are simply the product of the individual allele frequencies. The log likelihood under the null hypothesis, $\log L_0$, is calculated based on these haplotype frequencies with degrees of freedom $\text{df}_0 = \sum_{i=1}^{m}(k_i - 1)$, where m is the number of loci and k_i is the number of alleles for the ith locus (Zhao, Curtis, and Sham 2000). Under the alternative hypothesis, the log likelihood, $\log L_1$ is calculated from the EM estimates of the haplotype frequencies with degrees of freedom $\mathrm{df}_1 =$ number of haplotypes $- 1$. A likelihood ratio test is used to test this hypothesis as follows:

$$2(\log L_1 - \log L_0) \sim \chi^2_\nu$$

where $\nu = \mathrm{df}_1 - \mathrm{df}_0$ is the difference between the number of degrees of freedom under the null hypothesis and the alternative.

Testing for Trait Associations

When the TRAIT statement is included in PROC HAPLOTYPE, case-control tests are performed to test for association between the dichotomous trait (often, an indicator of individuals with or without a disease) and the marker loci using haplotypes. In addition to an omnibus test that is performed over all haplotypes, when the TESTALL option is specified in the TRAIT statement, a test for association between each individual haplotype and the trait is performed. Note that the individual haplotype tests should only be performed if the omnibus test statistic is significant.

Chi-Square Tests

The test performed over all haplotypes is based on the log likelihoods: under the null hypothesis, the log likelihood over all the individuals in the sample, regardless of the value of their trait variable, is calculated as described in the section "The EM Algorithm" on page 95; the log likelihood is also calculated separately for the two sets of individuals within the sample as determined by the trait value under the alternative hypothesis of marker-trait association. A likelihood ratio test (LRT) statistic can then be formed as follows:

$$X^2 = 2(\log L_1 + \log L_2 - \log L_0)$$

where $\log L_0$, $\log L_1$, and $\log L_2$ are the log likelihoods under the null hypothesis, for individuals with the first trait value, and for individuals with the second trait value, respectively (Zhao, Curtis, and Sham 2000). Defining degrees of freedom for each log likelihood similarly, this statistic has an asymptotic chi-square distribution with $(\mathrm{df}_1 + \mathrm{df}_2 - \mathrm{df}_0)$ degrees of freedom.

An association between individual haplotypes and the trait can also be tested. To do so, the following contingency table is formed:

Table 5.1. Haplotype-Trait Counts

	Hap 1	Hap 2	Total
Trait 1	c_{11}	c_{12}	t_1
Trait 2	c_{21}	c_{22}	t_2
Total	h_1	h_2	T

where $T = 2n = t_1 + t_2 = h_1 + h_2$, the total number of haplotypes in the sample, "Hap 1" refers to the current haplotype being tested, "Hap 2" refers to all other haplotypes, and c_{ij} is the pseudo-observed count of individuals with trait i and haplotype j (note that these counts are not necessarily integers since haplotypes are not actually observed; they are calculated based on the estimated haplotype frequencies). The column totals h_j are not calculated in the usual fashion, the sum of the cells in each column; rather, h_1 and h_2 are calculated as $T * f_j$ and $T - T * f_j$ respectively, where f_j is the estimated frequency of "Hap 1" in the overall sample.

The usual contingency table chi-square test statistic has a 1 df chi-square distribution:

$$\sum_{i=1,2} \sum_{j=1,2} \frac{(c_{ij} - t_i h_j / T)^2}{t_i h_j / T}$$

Permutation Tests

Since the assumption of a chi-square distribution in the preceding section may not hold, estimates of exact p-values via Monte Carlo methods are recommended. New samples are formed by randomly permuting the trait values, and either of the chi-square test statistics shown in the previous section can be calculated for each of these samples. The number of new samples created is determined by the number given in the PERMS= option of the TRAIT statement. The exact p-value approximation is then calculated as m/p, where m is the number of samples with a test statistic greater than or equal to the test statistic in the actual sample and p is the total number of permutation samples. This method is used to obtain empirical p-values for both the overall and the individual haplotype tests (Zhao, Curtis, and Sham 2000; Fallin et al. 2001).

Missing Values

An individual's m-locus genotype is considered to be partially missing if any, but not all, of the alleles are missing. Genotypes with all missing alleles are dropped. Also, if there are any markers with all missing values in a BY group (or the entire data set if there is no BY statement), no calculations are performed for that BY group. Partially missing genotypes are used in the EM algorithm and the jackknife procedure. In calculating the allele frequencies, missing alleles are dropped and the frequency of an allele u at a marker is obtained as the number of u alleles in the data divided by the total number of nonmissing alleles at the marker in the data. In the E-step of the EM algorithm, the frequency of a partially missing genotype is updated for every possible genotype. In the M-step, haplotypes resulting from a missing genotype may bear some missing alleles. Such a haplotype is not considered as a new haplotype, but rather all existing haplotypes that have alleles identical to the nonmissing alleles of this haplotype are updated. Dealing with missing genotypes involves looping through all possible genotypes in the E-step and all possible haplotypes in the M-step. The stepwise EM algorithm performs a series of two-step processes involving EM estimation followed by trimming the set of haplotypes. Thus, in the EM estimation step, missing values are handled as described for the EM algorithm. Depending on the input data set, missing genotypes can increase the computation time substantially for either estimation method.

When the TRAIT statement is specified, any observation with a missing trait value is dropped from calculations used in the tests for marker-trait association and haplotype-trait associations. However, observations with missing trait values are included in calculating the frequencies shown in the "Haplotype Frequencies" table, which are then used in the OUT= data set. The combined frequencies listed in the "Tests for Haplotype-Trait Association" table may therefore be different than these frequencies in this situation.

OUT= Data Set

The OUT= data set contains the following variables: the BY variables (if any), _ID_ that identifies the individual and/or any variables listed in the ID statement, the pair of alleles at each marker analyzed, HAPLOTYPE1 and HAPLOTYPE2 that contain the pair of haplotypes of which each genotype can be comprised, and PROB containing the probability of each individual's genotype being resolved into that haplotype pair. Note that when GENOCOL or the experimental option TALL is specified, the pair of alleles at a marker are contained in a single column separated by the delimiter '/' or the character given in the DELIMITER= option.

Displayed Output

This section describes the displayed output from PROC HAPLOTYPE. See the "ODS Table Names" section on page 102 for details about how this output interfaces with the Output Delivery System.

Analysis Information

The "Analysis Information" table lists information on the following settings used in PROC HAPLOTYPE:

- Loci Used, the loci used to form haplotypes
- Number of Individuals
- Number of Starts, the value specified in the NSTART= option or the default (1)
- Convergence Criterion, the value specified in the CONV= option or the default (0.00001)
- Iterations Checked for Conv., the value specified in the NLAG= option or the default (1)
- Maximum Number of Iterations, the value specified in the MAXITER= option or the default (100)
- Number of Iterations Used, as determined by the CONV= or MAXITER= option
- Log Likelihood, from the last iteration performed
- Initialization Method, the method specified in the INIT= option or "Linkage Equilibrium" by default

- Random Number Seed, the value specified in the SEED= option or generated by the system clock, or "Not Used" when a random seed is not used
- Standard Error Method, the method specified in the SE= option or "Binomial" by default
- Haplotype Frequency Cutoff, the value specified in the CUTOFF= option or the default (0)

Iteration History

The "Iteration History" table displays the log likelihood and the ratio of change for each iteration of the EM algorithm.

Haplotype Frequencies

The "Haplotype Frequencies" table lists all the possible m-locus haplotypes in the sample (where $2m$ variables are specified in the VAR statement), with an estimate of the haplotype frequency, the standard error of the frequency, and the lower and upper limits of the confidence interval for the frequency based on the confidence level determined by the ALPHA= option of the PROC HAPLOTYPE statement (0.95 by default). When the LD option is specified in the PROC HAPLOTYPE statement, haplotype frequency estimates are calculated both under the null hypothesis of no allelic association by taking the product of allele frequencies, and under the alternative, which allows for associations, using the EM algorithm.

Test for Allelic Associations

The "Test for Allelic Associations" table displays the degrees of freedom and log likelihood for the null hypothesis of no association and the alternative hypothesis of associations between markers. The chi-square statistic and its p-value are also shown for the test of these hypotheses.

Test for Marker-Trait Association

The "Test for Marker-Trait Association" table displays the number of observations, degrees of freedom, and log likelihood for both trait values as well as the combined sample. The chi-square test statistic and its corresponding p-value from performing the case-control test, testing the hypothesis of no association between the trait and the marker loci used in PROC HAPLOTYPE, are also given. When the PERMS= option is included in the TRAIT statement, estimates of exact p-values are provided as well.

Tests for Haplotype-Trait Association

The "Tests for Haplotype-Trait Association" table displays statistics from case-control tests performed on each individual haplotype when the TESTALL option is included in the TRAIT statement. A significant p-value indicates that there is an association between the haplotype and the trait. When the PERMS= option is also given in the TRAIT statement, estimates of exact p-values are provided as well.

ODS Table Names

PROC HAPLOTYPE assigns a name to each table it creates, and you must use this name to reference the table when using the Output Delivery System (ODS). These names are listed in the following table.

Table 5.2. ODS Tables Created by the HAPLOTYPE Procedure

ODS Table Name	Description	Statement or Option
AnalysisInfo	Analysis information	default
IterationHistory	Iteration history	ITPRINT
ConvergenceStatus	Convergence status	default
HaplotypeFreq	Haplotype frequencies	default
LDTest	Test for allelic associations	LD
CCTest	Test for marker-trait association	TRAIT statement
HapTraitTest	Tests for haplotype-trait association	TRAIT / TESTALL

Examples

Example 5.1. Estimating Three-Locus Haplotype Frequencies

Here is an example of 227 individuals genotyped at three markers, data which were created based on genotype frequency tables from the Lab of Statistical Genetics at Rockefeller University (2001). Note that when reading in the data, there are four individuals' genotypes per line, except for the last line of the DATA step, which contains three individuals' genotypes. The SAS data set that is created contains one individual per row with six columns representing the two alleles at each of three marker loci.

```
data ehdata;
   input m1-m6 @@;
   datalines;
1 1 1 1 1 3 1 1 1 1 1 3 1 1 1 1 1 3 1 1 1 1 1 3
1 1 1 1 1 3 1 1 1 1 1 3 1 1 1 1 1 3 1 1 1 1 1 3
1 1 1 1 1 3 1 1 1 1 1 3 1 1 1 1 1 3 1 1 1 1 1 3
1 1 1 1 2 3 1 1 1 1 2 3 1 1 1 1 2 3 1 1 1 1 3 3
1 1 1 1 3 3 1 1 1 1 3 3 1 1 1 1 3 3 1 1 1 1 3 3
1 1 1 1 3 3 1 1 1 1 3 3 1 1 1 1 3 3 1 1 1 1 3 3
1 1 1 2 1 1 1 1 1 2 1 1 1 1 1 2 1 2 1 1 1 2 1 2
1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2
1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2
1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 2 2 2 1 1 1 2 2 2
1 1 1 2 1 3 1 1 1 2 1 3 1 1 1 2 2 3 1 1 1 2 2 3
1 1 1 2 2 3 1 1 1 2 3 3 1 1 1 2 3 3 1 1 1 2 3 3
1 1 1 2 3 3 1 1 2 2 1 1 1 1 2 2 1 1 1 1 2 2 1 1
1 1 2 2 1 1 1 1 2 2 1 1 1 1 2 2 1 2 1 1 2 2 1 2
1 1 2 2 1 2 1 1 2 2 1 2 1 1 2 2 2 2 1 1 2 2 2 2
```

```
1 1 2 2 2 2 1 1 2 2 2 2 1 1 2 2 1 3 1 1 2 2 1 3
1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3
1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3
1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3
1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3
1 1 2 2 3 3 1 1 2 2 3 3 1 2 1 1 1 1 1 2 1 1 1 1
1 2 1 1 1 1 1 2 1 1 1 1 1 2 1 1 1 1 1 2 1 1 1 1
1 2 1 1 1 1 1 2 1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 3
1 2 1 1 1 3 1 2 1 1 2 3 1 2 1 1 2 3 1 2 1 1 2 3
1 2 1 1 2 3 1 2 1 1 2 3 1 2 1 1 2 3 1 2 1 1 3 3
1 2 1 1 3 3 1 2 1 1 3 3 1 2 1 2 1 1 1 2 1 2 1 1
1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2 1 1
1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2 1 1 1 2 1 2 1 3
1 2 1 2 1 3 1 2 1 2 1 3 1 2 1 2 2 3 1 2 1 2 2 3
1 2 1 2 2 3 1 2 1 2 2 3 1 2 1 2 3 3 1 2 1 2 3 3
1 2 2 2 1 1 1 2 2 2 1 1 1 2 2 2 1 1 1 2 2 2 1 1
1 2 2 2 1 1 1 2 2 2 1 1 1 2 2 2 1 1 1 2 2 2 1 1
1 2 2 2 1 1 1 2 2 2 1 1 1 2 2 2 1 2 1 2 2 2 1 2
1 2 2 2 1 2 1 2 2 2 1 3 1 2 2 2 1 3 1 2 2 2 2 3
1 2 2 2 2 3 1 2 2 2 2 3 1 2 2 2 3 3 1 2 2 2 3 3
1 2 2 2 3 3 1 2 2 2 3 3 1 2 2 2 3 3 1 2 2 2 3 3
1 2 2 2 3 3 1 2 2 2 3 3 2 2 1 1 1 1 2 2 1 1 1 2
2 2 1 1 1 2 2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1 2 2
2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1 2 2
2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1 1 3
2 2 1 1 1 3 2 2 1 1 2 3 2 2 1 1 2 3 2 2 1 1 2 3
2 2 1 1 2 3 2 2 1 1 2 3 2 2 1 1 2 3 2 2 1 1 2 3
2 2 1 1 2 3 2 2 1 1 2 3 2 2 1 1 3 3 2 2 1 1 3 3
2 2 1 1 3 3 2 2 1 1 3 3 2 2 1 2 1 1 2 2 1 2 1 1
2 2 1 2 1 1 2 2 1 2 1 1 2 2 1 2 1 1 2 2 1 2 1 2
2 2 1 2 1 2 2 2 1 2 1 2 2 2 1 2 2 2 2 2 1 2 2 2
2 2 1 2 2 2 2 2 1 2 2 2 2 2 1 2 1 3 2 2 1 2 1 3
2 2 1 2 1 3 2 2 1 2 1 3 2 2 1 2 1 3 2 2 1 2 1 3
2 2 1 2 2 3 2 2 1 2 2 3 2 2 1 2 2 3 2 2 1 2 2 3
2 2 1 2 2 3 2 2 1 2 2 3 2 2 1 2 2 3 2 2 1 2 3 3
2 2 1 2 3 3 2 2 1 2 3 3 2 2 2 2 1 1 2 2 2 2 1 1
2 2 2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1
2 2 2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 1 2 2 2 2 1 2
2 2 2 2 1 2 2 2 2 2 1 2 2 2 2 2 2 3 2 2 2 2 2 3
2 2 2 2 2 3 2 2 2 2 3 3 2 2 2 2 3 3 2 2 2 2 3 3
2 2 2 2 3 3 2 2 2 2 3 3 2 2 2 2 3 3 2 2 2 2 3 3
2 2 2 2 3 3 2 2 2 2 3 3 2 2 2 2 3 3
;
```

The haplotype frequencies can be estimated using the EM algorithm and their standard errors estimated using the jackknife method by implementing the following code:

```
proc haplotype data=ehdata se=jackknife maxiter=20 itprint nlag=4;
   var m1-m6;
run;
```

This produces the following ODS output:

Output 5.1.1. Analysis Information for the HAPLOTYPE Procedure

```
                          The HAPLOTYPE Procedure

                           Analysis Information

               Loci Used                                     M1 M2 M3
               Number of Individuals                              227
               Number of Starts                                     1
               Convergence Criterion                          0.00001
               Iterations Checked for Conv.                         4
               Maximum Number of Iterations                        20
               Number of Iterations Used                           11
               Log Likelihood                              -934.97918
               Initialization Method            Linkage Equilibrium
               Standard Error Method                        Jackknife
               Haplotype Frequency Cutoff                           0
```

Output 5.1.1 displays information on several of the settings used to perform the HAPLOTYPE procedure on the ehdata data set. Note that though the MAXITER= option was set to 20 iterations, convergence according to the criterion of 0.00001 was reached for four consecutive iterations prior to the 20th iteration, at which point the estimation process stopped. To obtain more precise frequency estimates, a lower convergence criterion can be used.

Output 5.1.2. Iteration History for the HAPLOTYPE Procedure

```
                              Iteration History

                                                Ratio
                        Iter        LogLike    Changed

                           0    -953.89697
                           1    -937.92181    0.01675
                           2    -935.91870    0.00214
                           3    -935.35775    0.00060
                           4    -935.13050    0.00024
                           5    -935.03710    0.00010
                           6    -935.00051    0.00004
                           7    -934.98679    0.00001
                           8    -934.98180    0.00001
                           9    -934.98002    0.00000
                          10    -934.97940    0.00000
                          11    -934.97918    0.00000
```

Output 5.1.3. Convergence Status for the HAPLOTYPE Procedure

```
         Algorithm converged.
```

Because the ITPRINT option was specified in the PROC HAPLOTYPE statement, the iteration history of the EM algorithm is included in the ODS output. Output 5.1.2

contains the table displaying this information. By default, the “Convergence Status” table is displayed (Output 5.1.3), which only consists of one line indicating whether convergence was met.

Output 5.1.4. Haplotype Frequencies from the HAPLOTYPE Procedure

```
                              Haplotype Frequencies

                                             Standard      95% Confidence
      Number     Haplotype         Freq         Error          Limits

           1     1-1-1          0.09170       0.01505     0.06221     0.12119
           2     1-1-2          0.02080       0.00952     0.00214     0.03946
           3     1-1-3          0.11509       0.01766     0.08048     0.14971
           4     1-2-1          0.07904       0.01696     0.04580     0.11228
           5     1-2-2          0.06768       0.01546     0.03738     0.09799
           6     1-2-3          0.12788       0.02094     0.08685     0.16891
           7     2-1-1          0.05521       0.01227     0.03115     0.07926
           8     2-1-2          0.11700       0.01782     0.08207     0.15193
           9     2-1-3          0.07376       0.01495     0.04446     0.10307
          10     2-2-1          0.11766       0.01831     0.08177     0.15355
          11     2-2-2          0.03020       0.00899     0.01257     0.04782
          12     2-2-3          0.10397       0.01833     0.06805     0.13989
```

Output 5.1.4 displays the 12 possible three-locus haplotypes in the data and their estimated haplotype frequencies, standard errors, and bounds for the 95% confidence intervals for the estimates.

To see how the CUTOFF= option affects the “Haplotype Frequencies” table, suppose you want to view only the haplotypes with an estimated frequency of at least 0.10. The following code creates such a table:

```
proc haplotype data=ehdata se=jackknife cutoff=0.10 nlag=4;
  var m1-m6;
run;
```

Now, the “Haplotype Frequencies” table is displayed as:

Output 5.1.5. Haplotype Frequencies from the HAPLOTYPE Procedure Using the CUTOFF= Option

```
                            The HAPLOTYPE Procedure

                              Haplotype Frequencies

                                             Standard      95% Confidence
      Number     Haplotype         Freq         Error          Limits

           1     1-1-3          0.11509       0.01766     0.08048     0.14971
           2     1-2-3          0.12788       0.02094     0.08685     0.16891
           3     2-1-2          0.11700       0.01782     0.08207     0.15193
           4     2-2-1          0.11766       0.01831     0.08177     0.15355
           5     2-2-3          0.10397       0.01833     0.06805     0.13989
```

Output 5.1.5 displays only the five 3-locus haplotypes with estimated frequencies of at least 0.10. This option is especially useful for keeping the “Haplotype Frequencies” table to a manageable size when many marker loci or loci with several alleles are used, and many of the haplotypes have estimated frequencies very near zero. Using CUTOFF=1 suppresses the “Haplotype Frequencies” table.

Example 5.2. Using Multiple Runs of the EM Algorithm

Continuing the example from the section “Getting Started” on page 86, suppose you are concerned that the likelihood reached a local and not a global maximum. You can request that PROC HAPLOTYPE use several different sets of initial haplotype frequencies to ensure that you find a global maximum of the likelihood. The following code invokes the EM algorithm using five different sets of initial values, including the set used in the Getting Started example:

```
proc haplotype data=markers prefix=SNP init=random seed=51220
               nstart=5;
   var m1-m8;
run;
```

The NSTART=5 option requests that the EM algorithm be run three times using randomly generated initial frequencies, including once using the seed 51220 that was previously used, once using uniform initial frequencies, and once using haplotype frequencies given by the product of the allele frequencies. The following two tables are from the run that produced the best log likelihood:

Output 5.2.1. Output from PROC HAPLOTYPE

```
              The HAPLOTYPE Procedure

               Analysis Information

Loci Used                       SNP1 SNP2 SNP3 SNP4
Number of Individuals                            25
Number of Starts                                  5
Convergence Criterion                       0.00001
Iterations Checked for Conv.                      1
Maximum Number of Iterations                    100
Number of Iterations Used                        19
Log Likelihood                            -95.94742
Initialization Method                        Random
Random Number Seed                        499887544
Standard Error Method                      Binomial
Haplotype Frequency Cutoff                        0
```

Output 5.2.1. (continued)

```
                         Haplotype Frequencies

                                            Standard      95% Confidence
       Number     Haplotype         Freq       Error          Limits

            1     A-A-A-A        0.14324     0.05005     0.04515     0.24133
            2     A-A-A-B        0.07507     0.03764     0.00129     0.14885
            3     A-A-B-A        0.00000     0.00001     0.00000     0.00001
            4     A-A-B-B        0.00000     0.00010     0.00000     0.00019
            5     A-B-A-A        0.09295     0.04148     0.01165     0.17425
            6     A-B-A-B        0.05349     0.03214     0.00000     0.11649
            7     A-B-B-A        0.00001     0.00052     0.00000     0.00103
            8     A-B-B-B        0.07523     0.03768     0.00138     0.14909
            9     B-A-A-A        0.08644     0.04014     0.00776     0.16512
           10     B-A-A-B        0.08784     0.04044     0.00859     0.16710
           11     B-A-B-A        0.07904     0.03854     0.00350     0.15459
           12     B-A-B-B        0.10836     0.04441     0.02133     0.19540
           13     B-B-A-A        0.10097     0.04304     0.01661     0.18533
           14     B-B-A-B        0.00000     0.00000     0.00000     0.00000
           15     B-B-B-A        0.09735     0.04235     0.01435     0.18035
           16     B-B-B-B        0.00000     0.00000     0.00000     0.00000
```

Example 5.3. Testing for Linkage Disequilibrium

Again looking at the data from the Lab of Statistical Genetics at Rockefeller University (2001), if you request the test for linkage disequilibrium by specifying the LD option in the PROC HAPLOTYPE statement, the “Test for Allelic Associations” table containing the test statistics is included in the output.

```
proc haplotype data=ehdata ld;
   var m1-m6;
run;
```

The “Haplotype Frequencies” table now contains an extra column of the haplotype frequencies under the null hypothesis.

Output 5.3.1. Haplotype Frequencies Under the Null and Alternative Hypotheses

```
                           The HAPLOTYPE Procedure

                            Haplotype Frequencies

                                                     Standard       95% Confidence
Number     Haplotype     H0 Freq     H1 Freq           Error            Limits

     1     1-1-1         0.08172     0.09124         0.01353     0.06472     0.11775
     2     1-1-2         0.05605     0.02124         0.00677     0.00796     0.03452
     3     1-1-3         0.10006     0.11501         0.01499     0.08563     0.14439
     4     1-2-1         0.09084     0.07952         0.01271     0.05461     0.10443
     5     1-2-2         0.06231     0.06726         0.01177     0.04419     0.09032
     6     1-2-3         0.11122     0.12794         0.01569     0.09718     0.15870
     7     2-1-1         0.08100     0.05540         0.01075     0.03433     0.07647
     8     2-1-2         0.05556     0.11690         0.01510     0.08732     0.14649
     9     2-1-3         0.09918     0.07378         0.01228     0.04971     0.09785
    10     2-2-1         0.09005     0.11746         0.01513     0.08781     0.14711
    11     2-2-2         0.06176     0.03028         0.00805     0.01450     0.04606
    12     2-2-3         0.11025     0.10398         0.01434     0.07587     0.13209
```

Note that since the INIT= option was omitted from the PROC HAPLOTYPE statement, the initial haplotype frequencies used in the EM algorithm are identical to the frequencies that appear in the H0 FREQ column in Output 5.3.1. The frequencies in the H1 FREQ column are those calculated from the final iteration of the EM algorithm, and these frequencies' standard errors and confidence limits are included in the table as well.

Output 5.3.2. Testing for Linkage Disequilibrium Using the LD Option

```
                        Test for Allelic Associations

                                                                Chi-        Pr >
  Hypothesis                        DF         LogLike        Square       ChiSq

  H0: No Association                 4      -953.89697
  H1: Allelic Associations          11      -934.98180       37.8303      <.0001
```

Output 5.3.2 displays the log likelihood under the null hypothesis assuming independence among all the loci and the alternative, which allows for associations between markers. The empirical chi-square test statistic of the likelihood ratio test is calculated as $X^2 = 2[-934.98180 - (-953.89697)] = 37.8303$ with degrees of freedom $\nu = 11 - 4 = 7$ that gives a p-value < 0.0001. The test indicates significant linkage disequilibrium among the three loci, as shown in the online documentation from the Lab of Statistical Genetics at Rockefeller University (2001).

Example 5.4. Testing for Marker-Trait Associations

To demonstrate how the TRAIT statement can be utilized, a subset of data from GAW12 (Wijsman et al. 2001) is read into a SAS data set as follows:

```
data gaw;
   input status $ a1-a24;
   datalines;
U 8 4 4 4 2 7 3 2 1 4 10 2  6 6 1 2 1 1 7  7  8 7 8  8
U 5 9 3 5 3 4 2 3 4 3 14 10 3 6 7 7 1 4 5  12 3 3 1  2
A 8 2 5 1 6 3 3 5 3 4 5  3  3 1 5 3 3 4 7  7  7 3 7  7
U 7 8 5 3 8 4 5 3 3 4 13 8  1 3 4 5 4 4 10 7  1 2 2  2
U 9 2 2 5 7 6 9 3 2 4 3  2  5 2 1 2 2 4 5  7  4 3 1  12
U 2 7 1 4 6 7 8 4 4 3 10 5  5 2 4 3 3 1 8  11 2 3 7  7
U 7 7 6 6 1 4 9 5 3 1 14 6  5 3 1 3 3 1 12 1  3 7 7  7
U 4 4 3 7 3 2 8 9 3 1 9  10 6 4 5 3 1 4 10 8  8 5 8  2
A 8 9 6 5 6 4 3 4 4 1 9  1  7 7 2 5 4 1 1  1  5 1 10 2
U 9 5 6 1 2 6 3 3 3 2 8  7  1 5 3 8 1 3 1  8  3 5 1  4
U 8 1 1 5 8 6 3 3 4 3 1  10 3 1 2 3 4 4 5  10 4 5 7  9
A 7 2 3 4 1 3 2 3 3 3 7  1  7 7 2 3 3 4 5  1  5 5 7  9
U 9 3 1 1 2 3 9 8 3 1 13 13 7 1 2 2 3 4 10 3  1 1 10 1
U 2 9 6 1 3 4 3 2 4 3 2  1  4 3 8 1 4 3 9  5  4 2 1  10
U 2 1 1 4 4 7 5 8 3 4 10 13 5 4 4 4 4 3 12 2  3 7 2  12
U 7 7 6 6 3 3 9 3 4 3 14 14 2 1 2 2 1 4 9  1  5 8 4  10
U 1 3 6 5 5 4 9 4 3 4 13 1  2 3 1 2 1 3 1  3  5 3 2  1
U 9 2 6 6 3 4 3 4 2 4 14 9  5 2 4 4 1 1 12 7  5 5 11 7
U 3 3 5 5 8 4 6 5 4 3 2  13 7 1 1 2 3 2 10 7  3 4 7  10
U 4 3 4 5 7 7 8 8 3 3 8  13 3 4 3 2 4 1 1  12 1 3 10 7
U 3 8 1 1 3 8 8 3 4 4 13 12 1 4 5 7 1 4 1  8  3 2 3  3
U 7 8 5 7 7 3 3 3 4 3 14 5  5 1 8 5 4 4 12 12 5 5 10 10
A 7 2 5 4 1 3 3 9 4 3 13 9  2 3 6 5 4 4 1  10 5 2 1  10
U 7 2 4 5 6 1 1 2 4 4 10 8  4 5 5 4 1 1 6  9  2 7 2  12
U 3 3 4 2 7 3 8 3 4 4 14 12 3 2 5 4 3 3 9  3  2 1 12 12
A 2 3 4 1 4 3 3 3 4 4 6  14 1 1 2 2 1 3 3  1  2 8 2  7
U 5 9 3 1 7 4 3 4 2 4 9  8  5 7 3 1 1 3 9  9  2 5 1  9
U 8 5 6 5 3 7 4 4 4 3 10 9  7 5 2 8 4 1 7  8  2 7 12 1
U 9 8 5 5 7 3 6 5 1 3 13 5  2 2 8 7 3 3 9  12 1 3 4  1
A 7 8 5 2 3 5 3 9 3 3 12 5  1 1 1 2 1 4 7  2  5 3 6  1
A 5 4 1 1 3 7 4 5 3 3 14 13 7 3 3 1 4 3 1  8  3 3 2  9
U 8 9 3 2 7 3 8 9 4 1 1  12 5 4 4 6 3 4 2  7  5 2 3  10
A 9 2 3 5 3 3 2 3 2 3 14 13 6 1 3 1 4 3 3  2  3 1 1  7
A 2 5 7 5 6 7 9 4 3 4 14 13 5 1 2 3 4 4 2  10 3 1 12 12
U 7 2 3 1 1 3 4 4 3 4 2  8  5 3 4 6 3 3 10 12 8 3 2  1
A 7 5 1 5 3 3 9 2 3 3 10 6  1 7 2 4 4 4 10 9  1 8 7  3
U 3 2 5 5 4 3 3 5 1 3 1  1  5 2 1 2 3 3 10 3  3 3 10 4
A 3 2 5 5 8 5 3 7 4 3 2  14 5 5 3 3 3 4 11 1  6 2 1  10
A 2 7 5 5 3 2 9 4 3 3 1  7  7 5 4 7 4 1 12 7  2 3 12 9
A 5 7 2 3 7 3 3 3 3 4 9  2  4 1 2 7 1 4 6  1  2 1 7  7
U 7 4 3 4 5 3 3 8 3 3 2  8  4 6 7 7 4 1 3  1  2 4 12 1
U 7 8 5 4 4 7 9 9 4 3 5  13 7 1 4 4 4 4 9  8  8 3 3  10
U 2 8 4 5 3 7 3 4 3 3 8  14 6 4 6 2 3 4 7  1  3 3 3  10
U 6 8 1 3 6 7 5 4 3 4 1  12 3 7 8 4 3 4 12 12 4 7 12 6
A 8 7 3 1 3 6 4 4 3 3 4  10 6 5 8 1 1 4 1  10 2 2 5  2
```

```
U 2 8 6 6 4 8 4 3 4 3 9  1  1 1 2 3 4 4 2  6  2 3 9  7
U 9 8 4 3 7 3 8 4 4 3 8  8  6 6 4 5 3 4 5  5  1 8 10 1
U 9 3 5 1 8 6 5 3 3 2 13 2  3 5 8 2 1 3 1  10 3 3 10 12
U 2 9 1 6 7 4 9 9 4 1 8  1  3 2 5 8 4 4 3  1  3 3 12 7
U 8 8 6 2 3 2 2 4 3 4 6  12 3 1 7 2 4 4 5  9  2 3 1  10
;
```

This data set contains twelve markers. Suppose you are interested in testing three of the marker loci at a time for association with the trait (status in this case: “A” for affected or “U” for unaffected with a particular disease) over all of their haplotypes. That is, assuming the markers are numbered in the order they appear on the chromosome, haplotypes at marker loci 1 through 3 are analyzed, then haplotypes at marker loci 4 through 6 are analyzed, and so on. These tests may be performed in addition to, or in place of, single-marker case-control tests (see Chapter 3 for more information). In order to reduce the amount of SAS code needed for this analysis, a SAS macro can be used as follows:

```
%macro hap_trait;
 %do firsta=1 %to 19 %by 6;
  %let lasta=%eval(&firsta+5);
  %let firstm=%eval((&firsta+1)/2);
  %let lastm=%eval(&lasta/2);
  title "Markers &firstm through &lastm";

  proc haplotype data=gaw noprint;
     var a&firsta-a&lasta;
     trait status;
  run;

 %end;
%mend;
%hap_trait
```

Since the NOPRINT option is specified, this code produces only the “Test for Marker-Trait Association” table each of the four times PROC HAPLOTYPE is invoked.

Output 5.4.1. Testing for Marker-Trait Associations Using Haplotypes

```
                                Markers 1 through 3

                              The HAPLOTYPE Procedure

                         Test for Marker-Trait Association

  Trait     Trait             Num                                  Chi-      Pr >
 Number     Value             Obs        DF        LogLike       Square     ChiSq

      1     U                  36       156     -245.18487
      2     A                  14        68      -69.90500
            Combined           50       181     -355.16139      80.1430    0.0005



                                Markers 4 through 6

                              The HAPLOTYPE Procedure

                         Test for Marker-Trait Association

  Trait     Trait             Num                                  Chi-      Pr >
 Number     Value             Obs        DF        LogLike       Square     ChiSq

      1     U                  36       140     -236.78471
      2     A                  14        62      -78.22280
            Combined           50       162     -349.30084      68.5867    0.0033



                                Markers 7 through 9

                              The HAPLOTYPE Procedure

                         Test for Marker-Trait Association

  Trait     Trait             Num                                  Chi-      Pr >
 Number     Value             Obs        DF        LogLike       Square     ChiSq

      1     U                  36       119     -242.53993
      2     A                  14        56      -68.34854
            Combined           50       139     -348.95917      76.1414    0.0001



                               Markers 10 through 12

                              The HAPLOTYPE Procedure

                         Test for Marker-Trait Association

  Trait     Trait             Num                                  Chi-      Pr >
 Number     Value             Obs        DF        LogLike       Square     ChiSq

      1     U                  36       180     -268.92245
      2     A                  14        75      -85.15400
            Combined           50       233     -395.70275      83.2526    <.0001
```

Output 5.4.1 displays the four tables that are created by this macro. The first corresponds to testing the three-locus haplotypes at the first three marker loci with the

TRAIT variable, the second to the second set of three markers, and so on. From the LRTs that are performed and summarized in the output, it can be concluded that out of the four sets of marker loci tested, the haplotypes at markers 10, 11, and 12 show the most significant association with the trait variable status. The chi-square statistic for testing the haplotypes at these markers for association with disease status is calculated as $83.2526 = 2(-268.92245 - 85.15400 + 395.70275)$ with degrees of freedom $22 = 180 + 75 - 233$, which has a p-value < 0.0001.

Suppose you would like to further explore the association between these three markers and the trait. You can also perform tests of association between each individual haplotype at these marker loci and disease status using the following code:

```
ods output haplotype.haptraittest=outhap;
proc haplotype data=gaw noprint;
   var a19-a24;
   trait status / testall perms=100;
run;

proc print data=outhap(obs=20) noobs;
   title 'The HAPLOTYPE Procedure';
   title2 ' ';
   title3 'Tests for Haplotype-Trait Association';
run;
ods output close;
```

The TESTALL option indicates that a test for trait association should be performed on each haplotype using a chi-square test statistic, which is performed by default. In addition, since the PERMS=100 option is included, an empirical p-value is calculated. Due to the number of alleles at each marker in this example, this option increases the computation time substantially, even with this small number of permutations.

Output 5.4.2. Using the TESTALL Option on Markers 10-12

```
                           The HAPLOTYPE Procedure

                     Tests for Haplotype-Trait Association

                                         Combined                  Prob     Prob
Number  Haplotype  Trait1Freq  Trait2Freq    Freq          ChiSq   ChiSq    Exact

     1   1-1-2       0.00000     0.03571   0.00000             0  1.0000   1.0000
     2   1-1-7       0.00000     0.00000   0.01000        1.0101  0.3149   0.2800
     3   1-1-10      0.00000     0.00000   0.01950        1.9883  0.1585   0.2900
     4   1-2-1       0.00000     0.01786   0.03000        2.3686  0.1238   0.1300
     5   1-2-2       0.00000     0.05357   0.01000        6.0967  0.0135   0.0300
     6   1-2-3       0.00000     0.00000   0.00000      0.001666  0.9674   0.5900
     7   1-2-5       0.00000     0.00000   0.01000        1.0101  0.3149   0.2300
     8   1-2-7       0.00000     0.05357   0.00000             0  1.0000   1.0000
     9   1-2-10      0.00000     0.01786   0.00000             0  1.0000   1.0000
    10   1-2-12      0.00000     0.00000   0.00000             0  1.0000   1.0000
    11   1-3-1       0.00694     0.00000   0.00000             0  1.0000   1.0000
    12   1-3-2       0.00000     0.01786   0.01000        0.9019  0.3423   0.4600
    13   1-3-3       0.02777     0.00000   0.02000        0.7934  0.3731   0.7200
    14   1-3-4       0.00000     0.00000   0.00000             0  1.0000   1.0000
    15   1-3-7       0.04167     0.00000   0.02045        2.2035  0.1377   0.1100
    16   1-3-9       0.00000     0.01786   0.00000             0  1.0000   1.0000
    17   1-3-10      0.00000     0.00000   0.00000     7.8011E-8  0.9998   0.9200
    18   1-3-12      0.01389     0.00000   0.01006        0.3905  0.5320   0.9100
    19   1-4-1       0.01389     0.00000   0.00000             0  1.0000   1.0000
    20   1-4-12      0.00000     0.00000   0.00000             0  1.0000   1.0000
```

Output 5.4.2 displays the table "Test for Haplotype-Trait Association" as a SAS data set using the ODS system in order to show only the first 20 rows. The table contains haplotypes at markers 10, 11, and 12 and their estimated frequencies among individuals with the first trait value, individuals with the second trait value, and all individuals. The chi-square statistic testing whether the frequencies between the two trait groups are significantly different is also shown, along with its 1 df p-value. Note that none of the haplotypes shown here have an association with disease status significant at the 0.05 level according to the approximations of exact p-values.

Example 5.5. Creating a Data Set for a Regression Model

Another approach to testing haplotypes for association with a phenotype uses a regression model, which can be more powerful than the omnibus chi-square test performed in PROC HAPLOTYPE (Schaid et al. 2002; Zaykin et al. 2002). The output data set produced by PROC HAPLOTYPE can easily be transformed into one that can be used by one of the regression procedures offered by SAS/STAT. This approach can be used for quantitative traits as well as binary or ordinal traits.

Here is an example data set that can be analyzed using PROC HAPLOTYPE:

```
data alleles;
   input (a1-a6) ($) disease;
   datalines;
A  a  B  B  c  C  1
A  A  B  b  c  C  1
a  A  B  b  c  c  0
A  A  B  B  c  C  1
A  A  b  B  c  C  1
A  A  B  b  C  c  0
A  a  b  B  C  c  1
A  A  b  B  C  c  1
A  a  B  B  c  c  1
a  a  B  b  c  c  0
A  A  B  B  C  C  1
A  A  B  B  c  c  1
a  A  b  b  c  c  0
A  A  B  B  c  c  1
A  A  b  b  c  c  0
A  A  b  B  c  C  0
A  A  B  b  c  C  1
A  a  b  B  c  c  1
A  a  B  B  c  C  1
A  A  b  b  C  C  0
A  A  B  B  C  C  1
A  A  b  B  C  c  1
A  A  b  B  c  C  1
a  A  B  b  C  c  0
A  a  B  B  C  C  0
A  A  B  B  C  c  1
A  A  B  b  C  c  0
A  A  B  B  c  C  1
a  A  B  b  C  C  1
A  a  B  b  C  c  1
A  A  B  b  c  C  1
A  a  B  B  c  c  1
A  A  B  b  C  c  1
a  A  B  b  C  c  1
A  A  B  b  C  C  1
A  a  B  B  C  C  1
a  A  B  b  C  c  0
a  A  b  B  C  C  0
A  A  B  b  c  C  1
a  A  B  b  c  c  0
A  A  B  B  C  C  0
A  A  B  B  c  c  1
A  a  B  B  C  c  1
;
```

An output data set containing individuals' probabilities of having particular haplotype pairs can be created, with the ID statement and OUTID option indicating that this data set include the disease variable from the input data set and a unique identifier for each individual assigned by PROC HAPLOTYPE, respectively. An omnibus test for association between the three markers and disease status is also performed.

```
proc haplotype data=alleles out=out outid;
   var a1-a6;
   trait disease;
   id disease;
run;
```

This code executes the omnibus marker-trait association test whose p-value is given by the chi-square distribution.

Output 5.5.1. Testing for an Overall Marker-Trait Association

```
                                The HAPLOTYPE Procedure

                           Test for Marker-Trait Association

 Trait     Trait              Num                                  Chi-      Pr >
Number     Value              Obs         DF        LogLike      Square     ChiSq

     1     1                   29          7      -68.11558
     2     0                   14          7      -37.28544
           Combined            43          7     -115.48338     20.1647    0.0052
```

Output 5.5.1 shows that there is a significant overall association between the markers and the trait, disease status. However, the more powerful score test for regression can be used to perform a test for additive effects of the marker haplotypes.

```
data out1;
   set out;
   haplotype=tranwrd(haplotype1,'-','_');

data out2;
   set out;
   haplotype=tranwrd(haplotype2,'-','_');

data outnew;
   set out1 out2;

proc sort data=outnew;
   by haplotype;
run;

data outnew2;
   set outnew;
   lagh=lag(haplotype);
   if haplotype ne lagh then num+1;
   hapname=compress("H"||num,' ');

proc sort data=outnew2;
   by _id_ hapname;
run;

data outt;
```

```
   set outnew2;
   by _id_ haplotype;
   if first.haplotype then totprob=prob/2;
   else totprob+prob/2;
   if last.haplotype;

proc transpose data=outt out=outreg(drop=_NAME_) ;
   id hapname;
   idlabel haplotype;
   var totprob;
   by _id_ disease;
run;

data htr;
   set outreg;
   array h{8};
   do i=1 to 8;
    if h{i}=. then h{i}=0;
   end;
   keep _id_ disease h1-h8;

proc print data=htr noobs round label;
run;



proc logistic data=htr descending;
   model disease = h1-h8 / selection=stepwise;
run;
```

This SAS code produces a data set htr from the output data set of PROC HAPLOTYPE that contains the variables needed to be able to perform a regression analysis. There is now one column for each possible haplotype in the sample, with each column containing the haplotype's frequency, or probability, within an individual.

Output 5.5.2. Regression Data Set

```
_ID_    disease    A_B_C    A_B_c    a_B_C    a_B_c    A_b_C    A_b_c    a_b_c    a_b_C

   1       1        0.29     0.21     0.21     0.29     0.00     0.00     0.00     0.00
   2       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
   3       0        0.00     0.27     0.00     0.23     0.00     0.23     0.27     0.00
   4       1        0.50     0.50     0.00     0.00     0.00     0.00     0.00     0.00
   5       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
   6       0        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
   7       1        0.22     0.00     0.13     0.15     0.15     0.13     0.22     0.00
   8       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
   9       1        0.00     0.50     0.00     0.50     0.00     0.00     0.00     0.00
  10       0        0.00     0.00     0.00     0.50     0.00     0.00     0.50     0.00
  11       1        1.00     0.00     0.00     0.00     0.00     0.00     0.00     0.00
  12       1        0.00     1.00     0.00     0.00     0.00     0.00     0.00     0.00
  13       0        0.00     0.00     0.00     0.00     0.00     0.50     0.50     0.00
  14       1        0.00     1.00     0.00     0.00     0.00     0.00     0.00     0.00
  15       0        0.00     0.00     0.00     0.00     0.00     1.00     0.00     0.00
  16       0        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  17       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  18       1        0.00     0.27     0.00     0.23     0.00     0.23     0.27     0.00
  19       1        0.29     0.21     0.21     0.29     0.00     0.00     0.00     0.00
  20       0        0.00     0.00     0.00     0.00     1.00     0.00     0.00     0.00
  21       1        1.00     0.00     0.00     0.00     0.00     0.00     0.00     0.00
  22       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  23       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  24       0        0.22     0.00     0.13     0.15     0.15     0.13     0.22     0.00
  25       0        0.50     0.00     0.50     0.00     0.00     0.00     0.00     0.00
  26       1        0.50     0.50     0.00     0.00     0.00     0.00     0.00     0.00
  27       0        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  28       1        0.50     0.50     0.00     0.00     0.00     0.00     0.00     0.00
  29       1        0.01     0.00     0.49     0.00     0.49     0.00     0.00     0.01
  30       1        0.22     0.00     0.13     0.15     0.15     0.13     0.22     0.00
  31       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  32       1        0.00     0.50     0.00     0.50     0.00     0.00     0.00     0.00
  33       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  34       1        0.22     0.00     0.13     0.15     0.15     0.13     0.22     0.00
  35       1        0.50     0.00     0.00     0.00     0.50     0.00     0.00     0.00
  36       1        0.50     0.00     0.50     0.00     0.00     0.00     0.00     0.00
  37       0        0.22     0.00     0.13     0.15     0.15     0.13     0.22     0.00
  38       0        0.01     0.00     0.49     0.00     0.49     0.00     0.00     0.01
  39       1        0.27     0.23     0.00     0.00     0.23     0.27     0.00     0.00
  40       0        0.00     0.27     0.00     0.23     0.00     0.23     0.27     0.00
  41       0        1.00     0.00     0.00     0.00     0.00     0.00     0.00     0.00
  42       1        0.00     1.00     0.00     0.00     0.00     0.00     0.00     0.00
  43       1        0.29     0.21     0.21     0.29     0.00     0.00     0.00     0.00
```

The data set shown in Output 5.5.2 can now be used in one of the regression procedures offered by SAS/STAT. In this example, since the trait is binary, the LOGISTIC procedure can be used to perform a regression on the variable disease. The REG procedure could be used in a similar manner to analyze a quantitative trait.

Output 5.5.3. PROC LOGISTIC Output

```
                              The LOGISTIC Procedure

                     Testing Global Null Hypothesis: BETA=0

             Test                 Chi-Square       DF     Pr > ChiSq

             Likelihood Ratio         6.1962        1         0.0128
             Score                    6.3995        1         0.0114
             Wald                     4.9675        1         0.0258
```

Output 5.5.3. (continued)

```
                    Analysis of Maximum Likelihood Estimates

                                         Standard          Wald
       Parameter    DF     Estimate         Error    Chi-Square    Pr > ChiSq

       Intercept     1       1.1986        0.4058        8.7224        0.0031
       H8            1      -6.3249        2.8378        4.9675        0.0258
```

Output 5.5.3 shows two of the tables produced by PROC LOGISTIC. The first one displays the test of the global null hypothesis, $\beta = \mathbf{0}$. You can see that the score test indicates a significant association between the haplotypes at the three markers and disease status. In particular, the second table shows that as a result of the stepwise selection, the haplotype H8 (a-b-c) has a statistically significant effect on disease status. This is an example of how a regression analysis can be used to detect association in a similar manner to the LRT implemented by PROC HAPLOTYPE.

Example 5.6. Using the Tall-Skinny Data Format

This example uses the data from the example Testing for Marker-Trait Associations, with the data now in the tall-skinny format. When this format is used, BY groups can be created in order to estimate haplotype frequencies in nonoverlapping windows of marker loci instead of using a macro as shown in the other example; here four sets of three loci are examined, but in general, loci with the same value of the BY variable are included in the same analysis, so sets of varying sizes can be used as well.

```
data gaw_tall;
   input hap_win markername $ id status $ allele1 allele2;
   datalines;
 1        marker1        1      U          8          4
 1        marker1        2      U          5          9
 1        marker1        3      A          8          2
 1        marker1        4      U          7          8
 1        marker1        5      U          9          2
 1        marker1        6      U          2          7
 1        marker1        7      U          7          7

  ...

 4        marker12      42      U           3          10
 4        marker12      43      U           3          10
 4        marker12      44      U          12           6
 4        marker12      45      A           5           2
 4        marker12      46      U           9           7
 4        marker12      47      U          10           1
 4        marker12      48      U          10          12
 4        marker12      49      U          12           7
 4        marker12      50      U           1          10
;
```

Using the experimental options TALL, MARKER=, and INDIV=, along with the BY statement to indicate the BY variable representing haplotype windows, the same analysis shown in Testing for Marker-Trait Associations can be carried out on the 50 individuals typed at 12 markers, where sets of three loci at a time are tested for an association with the trait.

```
proc haplotype data=gaw_tall tall marker=markername indiv=id noprint;
   var allele1 allele2;
   by hap_win;
   trait status;
 run;
```

This produces the following ODS output, with Output 5.6.1 mirroring the results shown in Output 5.4.1 and hap_win=1 corresponding to the first three loci (‘Marker1’ through ‘Marker3’), and so on.

Output 5.6.1. Marker-Association Tests over Haplotype Windows

```
---------------------------------- hap_win=1 -----------------------------------

                            The HAPLOTYPE Procedure

                       Test for Marker-Trait Association

   Trait     Trait          Num                                Chi-       Pr >
  Number     Value          Obs        DF       LogLike      Square      ChiSq

       1     U               36       156    -245.18487
       2     A               14        68     -69.90500
             Combined        50       181    -355.16139     80.1430     0.0005



---------------------------------- hap_win=2 -----------------------------------

                            The HAPLOTYPE Procedure

                       Test for Marker-Trait Association

   Trait     Trait          Num                                Chi-       Pr >
  Number     Value          Obs        DF       LogLike      Square      ChiSq

       1     U               36       140    -236.78471
       2     A               14        62     -78.22280
             Combined        50       162    -349.30084     68.5867     0.0033



---------------------------------- hap_win=3 -----------------------------------

                            The HAPLOTYPE Procedure

                       Test for Marker-Trait Association

   Trait     Trait          Num                                Chi-       Pr >
  Number     Value          Obs        DF       LogLike      Square      ChiSq

       1     U               36       119    -242.53993
       2     A               14        56     -68.34854
             Combined        50       139    -348.95917     76.1414     0.0001



---------------------------------- hap_win=4 -----------------------------------

                            The HAPLOTYPE Procedure

                       Test for Marker-Trait Association

   Trait     Trait          Num                                Chi-       Pr >
  Number     Value          Obs        DF       LogLike      Square      ChiSq

       1     U               36       180    -268.92245
       2     A               14        75     -85.15400
             Combined        50       233    -395.70275     83.2526     <.0001
```

References

Clayton, D. (2002), "SNPHAP: A Program for Estimating Frequencies of Large Haplotypes of SNPs," [http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt].

Excoffier, L. and Slatkin, M. (1995), "Maximum-Likelihood Estimation of Molecular Haplotype Frequencies in a Diploid Population," *Molecular Biology and Evolution,* 12, 921–927.

Fallin, D., Cohen, A., Essioux, L., Chumakov, I., Blumenfeld, M., Cohen, D., and Schork, N.J. (2001), "Genetic Analysis of Case/Control Data Using Estimated Haplotype Frequencies: Application to APOE Locus Variation and Alzheimer's Disease," *Genome Research,* 11, 143–151.

Fallin, D. and Schork, N.J. (2000), "Accuracy of Haplotype Frequency Estimation for Biallelic Loci, via the Expectation-Maximization Algorithm for Unphased Diploid Genotype Data," *American Journal of Human Genetics,* 67, 947–959.

Hawley, M.E. and Kidd, K.K. (1995), "HAPLO: A Program Using the EM Algorithm to Estimate the Frequencies of Multi-site Haplotypes," *Journal of Heredity,* 86, 409–411.

Lab of Statistical Genetics at Rockefeller University (2001), "User's Guide to the EH Program," [http://linkage.rockefeller.edu/ott/eh.htm].

Long, J.C., Williams, R.C., and Urbanek, M. (1995), "An E-M Algorithm and Testing Strategy for Multiple-Locus Haplotypes," *American Journal of Human Genetics,* 56, 799–810.

Schaid, D.J., Rowland, C.M., Tines, D.E., Jacobson, R.M., and Poland, G.A. (2002), "Score Tests for Association between Traits and Haplotypes when Linkage Phase is Ambiguous," *American Journal of Human Genetics,* 70, 425–434.

Wijsman, E.M., Almasy, L., Amos, C.I., Borecki, I., Falk, C.T., King, T.M., Martinez, M.M., Meyers, D., Neuman, R., Olson, J.M., Rich, S., Spence, M.A., Thomas, D.C., Vieland, V.J., Witte, J.S., and MacCluer, J.W. (2001), "Analysis of Complex Genetic Traits: Applications to Asthma and Simulated Data," *Genetic Epidemiology,* 21, S1–S853.

Zaykin, D.V., Westfall, P.H., Young, S.S., Karnoub, M.A., Wagner, M.J., and Ehm, M.G. (2002), "Testing Association of Statistically Inferred Haplotypes with Discrete and Continuous Traits in Samples of Unrelated Individuals," *Human Heredity,* 53, 79–91.

Zhao, J.H., Curtis, D., and Sham, P.C. (2000), "Model-Free Analysis and Permutation Tests for Allelic Associations," *Human Heredity,* 50, 133–139.

Chapter 6
The HTSNP Procedure (Experimental)

Chapter Contents

Chapter 6

The HTSNP Procedure (Experimental)

Overview

Single nucleotide polymorphism (SNP) is the most abundant form of genetic variation and accounts for about 90% of human DNA polymorphism. There is roughly one SNP per 1kb in the human genome. Studies of human haplotype variations using SNPs over large genomic regions suggest the presence of discrete blocks with limited haplotype diversity punctuated by recombination hot spots. The intrablock linkage disequilibrium (LD) decreases only gradually with distance, while the interblock LD decays much more rapidly. Within each block, because of high LD, some allele(s) may always be coexistent with a particular allele at another locus such that (1) little haplotype diversity exists in the block, and (2) not all SNPs will be essential in characterizing the haplotype structure in the block. Therefore, the most common haplotypes could usually be captured by a small subset of SNPs, termed *haplotype tag SNPs (htSNPs)* by Johnson et al. (2001).

The selection of such a SNP subset that distinguishes all haplotypes, however, is known as the *minimum test set* problem and is NP-complete. The search space of choosing k SNPs out of m is $\binom{m}{k} = \frac{m!}{k!(m-k)!}$, for which enumerating all possible k-SNP combinations becomes impractical even for moderate numbers of m and k. The HTSNP procedure implements some heuristic algorithms for fast identification of an optimal subset of SNPs without mining through all possible combinations. An exhaustive search algorithm throughout the $\binom{m}{k}$ search space is also provided in PROC HTSNP.

Getting Started

Example

The following haplotypes from markers at the *CTLA4* locus (Johnson et al. 2001) can be read into a SAS data set as follows:

```
data ctla4;
   input (m1-m12)($) freq;
   datalines;
C T A A G C C A C C A G 0.333
T T A G G C C G C T G G 0.224
T C A G G C C G C T G G 0.058
T T A A G C C G C T G G 0.020
C T A A G T C A C C A G 0.080
C T A G G T C A C C A G 0.017
C T A G G C C A C C A G 0.045
T T A G G C C A C C A G 0.018
C T G G A C T A T C G A 0.086
C T G G A C C A T C G A 0.054
C T G G A C C A C C G A 0.021
;
```

You can now use PROC HTSNP to search a subset of markers that explains most of the haplotype richness in this sample. The following statements perform the search:

```
proc htsnp data=ctla4 size=5 method=im outstat=out
           cutoff=0.05 seed=244 conv=0.99;
   var m1-m12;
   freq freq;
run;

proc print data=out;
run;
```

The iterative maximization algorithm is selected as the search method with the METHOD=IM option. The SIZE=5 option indicates that only subsets containing exactly five SNPs are considered in the search. All haplotypes in the data set with a frequency below 0.05 are excluded from the search process since the CUTOFF=0.05 option was specified. The search continues until the convergence criterion of 0.99 is met as specified in the CONV= option. The iterative maximization algorithm randomly selects an initial set of markers, so using different seeds may produce different results.

The results from the procedure are as follows:

```
                          The HTSNP Procedure

                            Marker Summary

         Locus     Allele      Frequency    Diversity

         m1        C             0.66527      0.44537
         m1        T             0.33473      0.44537
         m2        C             0.06067      0.11398
         m2        T             0.93933      0.11398
         m3        A             0.83159      0.28010
         m3        G             0.16841      0.28010
         m4        A             0.45293      0.49557
         m4        G             0.54707      0.49557
         m5        A             0.16841      0.28010
         m5        G             0.83159      0.28010
         m6        C             0.89854      0.18234
         m6        T             0.10146      0.18234
         m7        C             0.91004      0.16373
         m7        T             0.08996      0.16373
         m8        A             0.68410      0.43221
         m8        G             0.31590      0.43221
         m9        C             0.85356      0.25000
         m9        T             0.14644      0.25000
         m10       C             0.68410      0.43221
         m10       T             0.31590      0.43221
         m11       A             0.51569      0.49951
         m11       G             0.48431      0.49951
         m12       A             0.16841      0.28010
         m12       G             0.83159      0.28010
```

Figure 6.1. Marker Summary for PROC HTSNP

Figure 6.1 displays the summary of the marker loci for this sample. This includes the frequency of each allele and the gene diversity at each marker.

```
      Obs    HTSNP1    HTSNP2    HTSNP3    HTSNP4    HTSNP5    PDE

       1       m2        m3        m6        m7        m8       1
```

Figure 6.2. OUTSTAT= Data set for PROC HTSNP

Figure 6.2 displays the output data set created, since the OUTSTAT= option was specified. This data set contains the set of five SNPs that were selected as the htSNPs; these five markers correspond to those selected by Johnson et al. (2001).

Syntax

The following statements are available in PROC HTSNP.

PROC HTSNP < *options* > **;**
  BY *variables* **;**
  FREQ *variable* **;**
  VAR *variables* **;**
  WEIGHT *variable* **;**

Items within angle brackets (< >) are optional, and statements following the PROC HTSNP statement can appear in any order. Only the VAR statement is required. The syntax for each statement is described in the following section in alphabetical order after the description of the PROC HTSNP statement.

PROC HTSNP Statement

PROC HTSNP < *options* > **;**

You can specify the following options in the PROC HTSNP statement.

BEST=*number*
specifies the number of the best selections stored in the output data set during exhaustive or simulated annealing search process when METHOD=SA or EX is specified. The *number* must be a positive integer. By default, only one best selection is reported.

CONV=*number*
specifies the convergence criterion for search of htSNPs, where $0 < number \leq 1$. The search process is stopped when the haplotype richness measurement is greater than or equal to *number* specified in the CONV= option. The default value is 0.90. When METHOD=SA or METHOD=EX is specified, the CONV= option is ignored and the searching continues until the annealing schedule is finished or the whole search space is traversed.

CUTOFF=*number*
specifies a lower bound on a haplotype's frequency in order for that haplotype to be included in evaluation of haplotype richness in the search process. The value of *number* must be between 0 and 1. By default, all haplotypes from the sample are included in the search process.

DATA=*SAS-data-set*
names the input SAS data set to be used by PROC HTSNP. The default is to use the most recently created data set.

MAXSIZE=*number*
specifies the maximum number of markers to be included in the subset for incremental search by default or when METHOD=INCR is specified. The number must be a positive integer that is less than or equal to the number of markers specified in the VAR statement. Searching is carried out until convergence is reached according to the convergence criterion, or *number* of markers have been included in the subset.

METHOD=INCR | INCREMENTAL
METHOD=DECR | DECREMENTAL
METHOD=EX | EXHAUSTIVE
METHOD=IM | ITERMAX
METHOD=SA | SIMANNEAL

indicates the method used for core marker set selection. By default or when METHOD=INCR is specified, the incremental search algorithm is used. When METHOD=DECR, the decremental algorithm is used. When METHOD=EX, the exhaustive search algorithm is used. When METHOD=IM, the iterative maximization algorithm is used. When METHOD=SA, the simulated annealing search algorithm is used.

NOPRINT

suppresses the display of the "Marker Summary" table.

OUTSTAT=*SAS-data-set*

names the output SAS data set containing the selections of htSNPs and the criterion for each selection. When this option is omitted, an output data set is created by default and named according to the DATA*n* convention.

SCHEDULE=*number*

specifies the number of reconfigurations used in each annealing step. The value for *number* must be a positive integer. The default value is 100 × (number of variables specified in the VAR statement).

SEED=*number*

specifies the initial seed for the random number generator used for the sampling of markers. The value for *number* must be an integer; the computer clock time is used if the option is omitted or an integer less than or equal to 0 is specified. For more details about seed values, refer to *SAS Language Reference: Concepts*.

SIZE=*number*

specifies the size of the subset of markers to select. The value for *number* must be a positive integer that is less than or equal to the number of markers specified in the VAR statement. The SIZE= option must be specified for an exhaustive search, iterative maximization search, and simulated annealing search.

STEP=*number*

specifies the steps used for simulated annealing search. The value for *number* must be a positive integer. The default value is 1.

TEMPERATURE=*number*
T=*number*

specifies the temperature used for the simulated annealing search when METHOD=SA is specified. The value for *number* must be a positive number. The default value is 1.

TFACTOR=*number*

specifies the factor by which the temperature is reduced for each annealing step during simulated annealing search. The value for *number* must satisfy $0 < number < 1$. The default value is 0.90.

BY Statement

BY *variables* **;**

You can specify a BY statement with PROC HTSNP to obtain separate analyses on observations in groups defined by the BY variables. When a BY statement appears, the procedure expects the input data set to be sorted in order of the BY variables. The *variables* are one or more variables in the input data set.

If your input data set is not sorted in ascending order, use one of the following alternatives:

- Sort the data using the SORT procedure with a similar BY statement.
- Specify the BY statement option NOTSORTED or DESCENDING in the BY statement for the HTSNP procedure. The NOTSORTED option does not mean that the data are unsorted but rather that the data are arranged in groups (according to values of the BY variables) and that these groups are not necessarily in alphabetical or increasing numeric order.
- Create an index on the BY variables using the DATASETS procedure (in base SAS software).

For more information on the BY statement, refer to the discussion in *SAS Language Reference: Concepts*. For more information on the DATASETS procedure, refer to the discussion in the *SAS Procedures Guide*.

FREQ Statement

FREQ *variable* **;**

The FREQ statement identifies the variable that indicates the frequency for each haplotype used in richness evaluation. If there is no FREQ statement, the frequency of each distinct haplotype is calculated by dividing its count by the total haplotype count. When a frequency value is missing or negative, the corresponding haplotype is ignored.

VAR Statement

VAR *variables* **;**

The VAR statement identifies the variables, one for each marker, containing the marker alleles that construct the haplotypes. Two or more variables must be specified.

WEIGHT Statement

WEIGHT *variable* **;**

The WEIGHT statement identifies the variable that indicates the weight for each haplotype used in richness evaluation. By default, all haplotypes are sampled with equal weights. When a weight value is missing or negative, the corresponding haplotype is ignored.

Details

Statistical Computations

Haplotype Richness Measurement

"Haplotype diversity" is calculated in PROC HTSNP to measure haplotype richness.

For n haplotypes, the haplotype diversity D_H records the weighted differences of all n^2 pairwise comparisons of two haplotypes (Clayton 2002).

$$D_H = \sum_{i=1}^{n} \sum_{j=1}^{n} w_i w_j (h_i - h_j)$$

where h_i and h_j denote the ith and the jth haplotype, and w_i and w_j are the corresponding weights. The difference of two m-locus haplotypes, h_i and h_j, is computed as the total allele differences at the m loci:

$$h_i - h_j = \sum_{k=1}^{m} (h_{ik} - h_{jk})$$

where h_{ik} is the allele of the ith haplotype observed at the kth locus and

$$h_{ik} - h_{jk} = \left\{ \begin{array}{ll} 0 & h_{ik} = h_{jk} \\ 1 & h_{ik} \neq h_{jk} \end{array} \right.$$

If only distinct haplotypes are recorded with their corresponding frequencies using the FREQ statement, then F_i, the weighted frequency of haplotype h_i, can be calculated as

$$F_i = \frac{w_i f_i}{\sum_j w_j f_j}$$

where w_j and f_j are the weight and frequency, respectively, of the jth haplotype. The estimate of haplotype diversity D_H is then proportional to the average of the gene diversity D_k at all m loci, computed as

$$D = \frac{\sum_{k=1}^{m} D_k}{m}$$

where $D_k = 1 - \sum_{u=1}^{l_k} p_{ku}^2$ and l_k is the number of alleles at the kth locus. The weighted allele frequency p_{ku} of the uth allele at the kth locus is recorded as

$$p_{ku} = \sum_{h_{ik}=u} F_i$$

The diversity computed in this way measures the probability that two haplotypes sampled from the population differ at any locus.

Goodness of a Core Set Selection

For a core set of k SNPs, the n observed haplotypes can be classified into G groups of k-locus haplotypes. The haplotype residual diversity, R_D, is defined as the sum of the within-group diversities for the G groups:

$$R_D = \sum_{g=1}^{G} F_g^2 \Big[\sum_{i \in G_g} \sum_{j \in G_g} F_{ig} F_{jg} (h_i - h_j) \Big]$$

where F_g is the weighted frequency of the gth group calculated as $F_g = \sum_{i \in G_g} F_i$ and F_{ig} is the within-group weighted frequency for the ith haplotype in the gth group with $F_{ig} = F_i / F_g$.

Similarly, R_D can be calculated as the total within-group gene diversity:

$$R_D = \sum_{g=1}^{G} F_g^2 D_g$$

and

$$D_g = \frac{\sum_{k=1}^{m} D_{kg}}{m}$$

where $D_{kg} = 1 - \sum_u p_{kug}^2$ is calculated using the within-group allele frequencies p_{kug}.

The proportion of diversity explained (PDE) by a SNP set selection is used to evaluate the goodness of that selection. PDE is calculated as

$$\text{PDE} = 1 - \frac{R_D}{D}$$

The selected search algorithm finds the optimal subset that maximizes PDE.

Incremental Search

The incremental search algorithm starts with finding a first marker that has maximum locus richness and then goes through the remaining markers to find the next one that brings in the greatest increase in PDE. The selected markers are kept and the search process is continued using the remaining ones, one marker being added at a time, until a convergence criterion is met.

Decremental Search

The decremental search operates in an opposite manner from the incremental search. Starting with all n markers, one marker that causes the smallest loss in PDE is excluded each time and the rest of the markers are kept. The exclusion process is continued until the PDE falls below a predefined criterion.

Iterative Maximization Search

The iterative maximization search (Gouesnard et al. 2001) is a fast algorithm for choosing an optimal k-subset from m accessions. The algorithm starts from a random selection of k markers for which all the core collections of size $k-1$ are tested. The subset with the highest PDE is retained. Among the other $m-k$ markers, one that brings the greatest increase in the goodness criterion is selected and a new k-locus set is obtained. Exclusion and inclusion of one marker in the new k-locus set is repeated until convergence. Each iteration needs to evaluate the PDE k times for $k-1$ markers and $m-k$ times for k markers.

Simulated Annealing Search

Simulation annealing (Kirkpatrick, Gelatt, and Vecchi 1983) has been adopted in many combinatorial optimization problems. The global optimum could be approximated with simulated annealing using a proper annealing schedule. Starting from a selection of k markers (the selection could be a random one or obtained from a previously mentioned algorithm), one marker is randomly swapped with another from the unselected markers. The change of haplotype goodness is evaluated using an energy function for the marker exchange. Acceptance of the exchange is judged with the Metropolis criterion (Metropolis et al. 1953), and

$$\Pr\{\text{new point is accepted}\} = \begin{cases} 1, & \Delta \le 0 \\ \exp(-\Delta/T), & \Delta > 0 \end{cases}$$

where Δ is the change of energy function and T is the annealing temperature.

Exhaustive Search

An exhaustive search of k markers from m involves traversal of all $\binom{m}{k}$ possible selections once and only once. The traversal is implemented in lexicographical order (Nijenhuis and Herbert 1978). Let $S_i = (s_1, s_2, ..., s_k)$ denote a selection i, where $1 \le s_{ij} \le m$ is the index of the jth element in selection i. Lexicographical traversal of all k subsets then starts with $(1, 2, ..., k-1, k)$, $(1, 2, ..., k-1, k+1)$, and ends with $(m-k+1, m-k+2, ..., m-1, m)$.

Missing Values

An m-locus haplotype is considered to be partially missing if any, but not all, of the alleles are missing. A haplotype that is all-missing is dropped for any analysis.

OUTSTAT= Data Set

The OUTSTAT= data set contains the following variables: the BY variables (if any); HTSNP1–HTSNPk that contain the names of the k selected haplotype tag markers; and the PDE variable that reports the PDE for each selection.

Displayed Output

This section describes the displayed output from PROC HTSNP. See the "ODS Table Names" section on page 134 for details about how this output interfaces with the Output Delivery System.

Marker Summary

The "Marker Summary" table lists the following information for each marker allele:

- Locus, the name of the marker locus
- Allele, the allele
- Frequency, the frequency of the allele
- Diversity, the gene diversity of the marker

Note that for the allele frequency and gene diversity calculations displayed in this table, the WEIGHT variable is ignored; the unweighted frequencies f_i are used.

ODS Table Names

PROC HTSNP assigns a name to each table it creates, and you must use this name to reference the table when using the Output Delivery System (ODS). These names are listed in the following table.

Table 6.1. ODS Tables Created by the HTSNP Procedure

ODS Table Name	Description	Statement or Option
MarkerSummary	Marker Summary	default

Example

Example 6.1. Using the HAPLOTYPE and HTSNP Procedures Together

Before using PROC HTSNP, you may need to first run PROC HAPLOTYPE (see Chapter 5, "The HAPLOTYPE Procedure" for more details) if you have data with unknown phase in order to estimate the haplotype frequencies. This example demonstrates how output from PROC HAPLOTYPE can be manipulated to be in the appropriate form for an input data set for PROC HTSNP.

The following data set contains 150 individuals with genotypes at 13 SNPs that were simulated to mimic the frequencies of SNPs in the *CASP8* gene (Johnson et al. 2001).

```
data casp8;
   input id (m1-m13) ($);
   datalines;
1 T/T T/T A/G G/G C/G A/G A/G G/C C/C G/G A/A A/G A/C
2 G/T T/T A/G T/G C/G G/G G/G C/C C/C G/G A/A A/G C/C
3 T/T C/T G/A G/G C/C G/A G/G C/G C/C G/G A/A A/A C/A

  ...

149 T/T T/T G/G G/G C/G G/G G/G C/C C/C G/G A/A A/G C/C
150 T/T T/T A/A G/G C/C A/A A/G G/G C/C G/G A/A A/A A/A
;
```

The following code can be used to first estimate haplotype frequencies using the EM algorithm, then to identify the haplotype tag SNPs.

```
ods output haplotypefreq=freqout(keep=haplotype freq);

proc haplotype data=casp8 genocol cutoff=0.0075;
   var m1-m13;
run;

data hapfreq;
   set freqout;
   array m{13} $ 1;
   do i = 1 to 13;
    m{i} = substr(haplotype, 2*i-1, 1);
   end;
   drop haplotype i;
run;

proc htsnp data=hapfreq size=4 method=sa best=5 cutoff=0.05
           seed=123 outstat=out;
   var m1-m13;
   freq freq;
run;

proc print data=out;
run;
```

The ODS statement is used to create a data set from the “Haplotype Frequencies” ODS table, which is displayed in its table form as follows:

Output 6.1.1. ODS Table Containing Haplotype Frequencies

```
                              The HAPLOTYPE Procedure

                                Haplotype Frequencies

                                                     Standard      95% Confidence
Number     Haplotype                          Freq       Error           Limits

     1     G-T-A-T-C-G-G-C-C-G-A-A-C    0.01988     0.00807     0.00406     0.03570
     2     T-C-G-G-C-G-G-C-C-G-A-A-C    0.09173     0.01669     0.05902     0.12445
     3     T-T-A-G-C-A-A-G-C-G-A-A-A    0.16666     0.02155     0.12442     0.20890
     4     T-T-A-G-C-A-G-G-C-G-A-A-A    0.05667     0.01337     0.03046     0.08287
     5     T-T-A-G-C-A-G-G-C-G-G-A-A    0.03663     0.01086     0.01534     0.05793
     6     T-T-G-G-C-A-G-G-C-G-A-A-A    0.01579     0.00721     0.00166     0.02992
     7     T-T-G-G-C-G-G-C-C-G-A-A-C    0.40576     0.02840     0.35011     0.46142
     8     T-T-G-G-C-G-G-G-T-C-A-A-A    0.02667     0.00932     0.00841     0.04493
     9     T-T-G-G-C-G-G-G-T-G-A-A-A    0.00861     0.00534     0.00000     0.01908
    10     T-T-G-G-G-G-G-C-C-G-A-G-C    0.16250     0.02133     0.12069     0.20432
```

With this table in the form of a SAS data set, the macro %PARSE can be used to convert it into an input data set for PROC HTSNP, using the estimated frequencies from PROC HAPLOTYPE as the FREQ variable. In this example, the simulated annealing search method is specified for finding the best sets of size four. The output data set OUT that is created by PROC HTSNP is then printed out to show the best five sets of SNPs that were selected.

Output 6.1.2. Output Data Set from PROC HTSNP

```
                Obs    HTSNP1    HTSNP2    HTSNP3    HTSNP4    PDE

                 1       m2        m5        m7        m13      1
                 2       m2        m7        m8        m12      1
                 3       m2        m5        m7        m8       1
                 4       m2        m7        m12       m13      1
                 5       m2        m5        m6        m7       1
```

Note that the last selection shown in Output 6.1.2 matches the set of htSNPs found by Johnson et al. (2001).

References

Clayton, D. (2002), "Choosing a Set of Haplotype Tagging SNPs from a Larger Set of Diallelic Loci," [http://www-gene.cimr.cam.ac.uk/clayton/software/stata/htSNP/htsnp.pdf].

Gouesnard, B., Bataillon, T.M., Decoux, G., Rozale, C., Schoen, D.J., and David, J.L. (2001), "MSTRAT: An Algorithm for Building Germ Plasm Core Collections by Maximizing Allelic or Phenotypic Richness," *The Journal of Heredity* 92(1), 93–94.

Johnson, G.C.L., Esposito, L., Barratt, B.J., Smith, A.N., Heward, J., Di Genova, G., Ueda, H., Cordell, H.J., Eaves, I.A., Dudbridge, F., Twells, R.C.J., Payne, F., Hughes, W., Nutland, S., Stevens, H., Carr, P., Tuomilehto-Wolf, E., Tuomilehto, J., Gough, S.C.L., Clayton, D.G., and Todd, J.A. (2001), “Haplotype Tagging for the Identification of Common Disease Genes,” *Nature Genetics* 29, 233–237.

Kirkpatrick, S., Gelatt, C.D., Jr., and Vecchi, M.P. (1983), “Optimization by Simulated Annealing,” *Science* 220, 671–680.

Metropolis, N., Rosenbluth, A., Rosenbluth, M., Teller, A., and Teller, E. (1953), “Equation of State Calculations by Fast Computing Machines,” *Journal of Chemical Physics* 21, 1087–1092.

Nijenhuis, A. and Herbert, S.W. (1978), *Combinatorial Algorithms for Computers and Calculators,* Second Edition, New York: Academic Press.

Chapter 7
The INBREED Procedure

Chapter Contents

Chapter 7
The INBREED Procedure

Overview

The INBREED procedure calculates the covariance or inbreeding coefficients for a pedigree. PROC INBREED is unique in that it handles very large populations.

The INBREED procedure has two modes of operation. One mode carries out analysis on the assumption that all the individuals belong to the same generation. The other mode divides the population into nonoverlapping generations and analyzes each generation separately, assuming that the parents of individuals in the current generation are defined in the previous generation.

PROC INBREED also computes averages of the covariance or inbreeding coefficients within sex categories if the sex of individuals is known.

Getting Started

This section demonstrates how you can use the INBREED procedure to calculate the inbreeding or covariance coefficients for a pedigree, how you can control the analysis mode if the population consists of nonoverlapping generations, and how you can obtain averages within sex categories.

For you to use PROC INBREED effectively, your input data set must have a definite format. The following sections first introduce this format for a fictitious population and then demonstrate how you can analyze this population using the INBREED procedure.

The Format of the Input Data Set

The SAS data set used as input to the INBREED procedure must contain an observation for each individual. Each observation must include one variable identifying the individual and two variables identifying the individual's parents. Optionally, an observation can contain a known covariance coefficient and a character variable defining the gender of the individual.

For example, consider the following data:

```
data Population;
   input Individual $ Parent1 $ Parent2 $
         Covariance Sex $ Generation;
   datalines;
   MARK   GEORGE LISA    .    M  1
   KELLY  SCOTT  LISA    .    F  1
   MIKE   GEORGE AMY     .    M  1
   .      MARK   KELLY  0.50  .  1
```

```
DAVID   MARK    KELLY    .     M  2
MERLE   MIKE    JANE     .     F  2
JIM     MARK    KELLY   0.50   M  2
MARK    MIKE    KELLY    .     M  2
;
```

It is important to order the pedigree observations so that individuals are defined before they are used as parents of other individuals. The family relationships between individuals cannot be ascertained correctly unless you observe this ordering. Also, older individuals must precede younger ones. For example, 'MARK' appears as the first parent of 'DAVID' at observation 5; therefore, his observation needs to be defined prior to observation 5. Indeed, this is the case (see observation 1). Also, 'DAVID' is older than 'JIM', whose observation appears after the observation for 'DAVID', as is appropriate.

In populations with distinct, nonoverlapping generations, the older generation (parents) must precede the younger generation. For example, the individuals defined in Generation=1 appear as parents of individuals defined in Generation=2.

PROC INBREED produces warning messages when a parent cannot be found. For example, 'JANE' appears as the second parent of the individual 'MERLE' even though there are no previous observations defining her own parents. If the population is treated as an overlapping population, that is, if the generation grouping is ignored, then the procedure inserts an observation for 'JANE' with missing parents just before the sixth observation, which defines 'MERLE' as follows:

```
JANE    .       .        .     F  2
MERLE   MIKE    JANE     .     F  2
```

However, if generation grouping is taken into consideration, then 'JANE' is defined as the last observation in Generation=1, as follows:

```
MIKE    GEORGE AMY       .     M  1
JANE    .       .        .     F  1
```

In this latter case, however, the observation for 'JANE' is inserted after the computations are reported for the first generation. Therefore, she does not appear in the covariance/inbreeding matrix, even though her observation is used in computations for the second generation (see the example on page 144).

If the data for an individual are duplicated, only the first occurrence of the data is used by the procedure, and a warning message is displayed to note the duplication. For example, individual 'MARK' is defined twice, at observations 1 and 8. If generation grouping is ignored, then this is an error and observation 8 is skipped. However, if the population is processed with respect to two distinct generations, then 'MARK' refers to two different individuals, one in Generation=1 and the other in Generation=2.

If a covariance is to be assigned between two individuals, then those individuals must be defined prior to the assignment observation. For example, a covariance of 0.50

can be assigned between ‘MARK’ and ‘KELLY’ since they are previously defined. Note that assignment statements must have different formats depending on whether the population is processed with respect to generations (see the “DATA= Data Set” section on page 150 for further information). For example, while observation 4 is valid for nonoverlapping generations, it is invalid for a processing mode that ignores generation grouping. In this latter case, observation 7 indicates a valid assignment, and observation 4 is skipped.

The latest covariance specification between any given two individuals overrides the previous one between the same individuals.

Performing the Analysis

To compute the covariance coefficients for the overlapping generation mode, use the following statements:

```
proc inbreed data=Population covar matrix init=0.25;
run;
```

Here, the DATA= option names the SAS data set to be analyzed, and the COVAR and MATRIX options tell the procedure to output the covariance coefficients matrix. If you omit the COVAR option, the inbreeding coefficients are output instead of the covariance coefficients.

Note that the PROC INBREED statement also contains the INIT= option. This option gives an initial covariance between any individual and unknown individuals. For example, the covariance between any individual and ‘JANE’ would be 0.25, since ‘JANE’ is unknown, except when ‘JANE’ appears as a parent (see Figure 7.1).

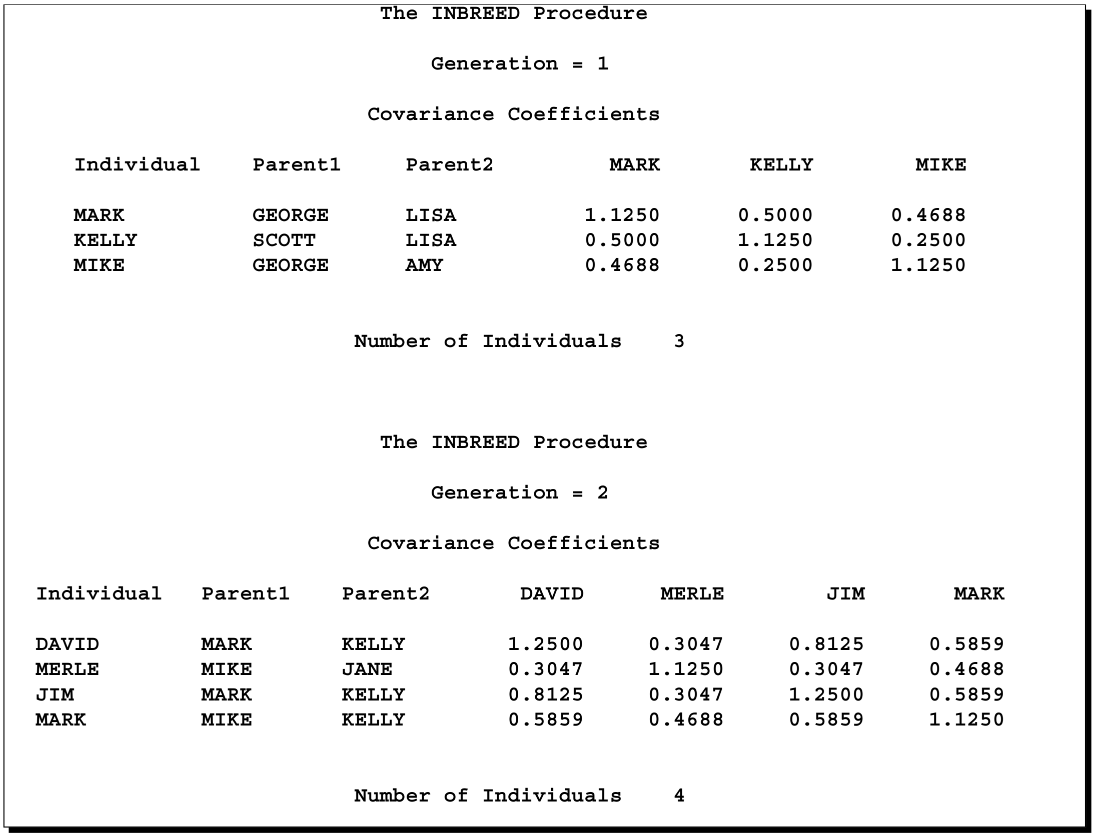
```
                              The INBREED Procedure

                             Covariance Coefficients

Individual  Parent1   Parent2     GEORGE       LISA       MARK      SCOTT      KELLY

GEORGE                            1.1250     0.2500     0.6875     0.2500     0.2500
LISA                              0.2500     1.1250     0.6875     0.2500     0.6875
MARK        GEORGE    LISA        0.6875     0.6875     1.1250     0.2500     0.5000
SCOTT                             0.2500     0.2500     0.2500     1.1250     0.6875
KELLY       SCOTT     LISA        0.2500     0.6875     0.5000     0.6875     1.1250
AMY                               0.2500     0.2500     0.2500     0.2500     0.2500
MIKE        GEORGE    AMY         0.6875     0.2500     0.4688     0.2500     0.2500
DAVID       MARK      KELLY       0.4688     0.6875     0.8125     0.4688     0.8125
JANE                              0.2500     0.2500     0.2500     0.2500     0.2500
MERLE       MIKE      JANE        0.4688     0.2500     0.3594     0.2500     0.2500
JIM         MARK      KELLY       0.4688     0.6875     0.8125     0.4688     0.8125

                             Covariance Coefficients

Individual  Parent1   Parent2        AMY       MIKE      DAVID       JANE      MERLE

GEORGE                            0.2500     0.6875     0.4688     0.2500     0.4688
LISA                              0.2500     0.2500     0.6875     0.2500     0.2500
MARK        GEORGE    LISA        0.2500     0.4688     0.8125     0.2500     0.3594
SCOTT                             0.2500     0.2500     0.4688     0.2500     0.2500
KELLY       SCOTT     LISA        0.2500     0.2500     0.8125     0.2500     0.2500
AMY                               1.1250     0.6875     0.2500     0.2500     0.4688
MIKE        GEORGE    AMY         0.6875     1.1250     0.3594     0.2500     0.6875
DAVID       MARK      KELLY       0.2500     0.3594     1.2500     0.2500     0.3047
JANE                              0.2500     0.2500     0.2500     1.1250     0.6875
MERLE       MIKE      JANE        0.4688     0.6875     0.3047     0.6875     1.1250
JIM         MARK      KELLY       0.2500     0.3594     0.8125     0.2500     0.3047

                             Covariance Coefficients

                    Individual  Parent1   Parent2        JIM

                    GEORGE                            0.4688
                    LISA                              0.6875
                    MARK        GEORGE    LISA        0.8125
                    SCOTT                             0.4688
                    KELLY       SCOTT     LISA        0.8125
                    AMY                               0.2500
                    MIKE        GEORGE    AMY         0.3594
                    DAVID       MARK      KELLY       0.8125
                    JANE                              0.2500
                    MERLE       MIKE      JANE        0.3047
                    JIM         MARK      KELLY       1.2500


                           Number of Individuals    11
```

Figure 7.1. Analysis for an Overlapping Population

In the previous example, PROC INBREED treats the population as a single generation. However, you may want to process the population with respect to distinct, nonoverlapping generations. To accomplish this, you need to identify the generation variable in a CLASS statement, as shown by the following statements.

```
proc inbreed data=Population covar matrix init=0.25;
   class Generation;
run;
```

Note that, in this case, the covariance matrix is displayed separately for each generation (see Figure 7.2).

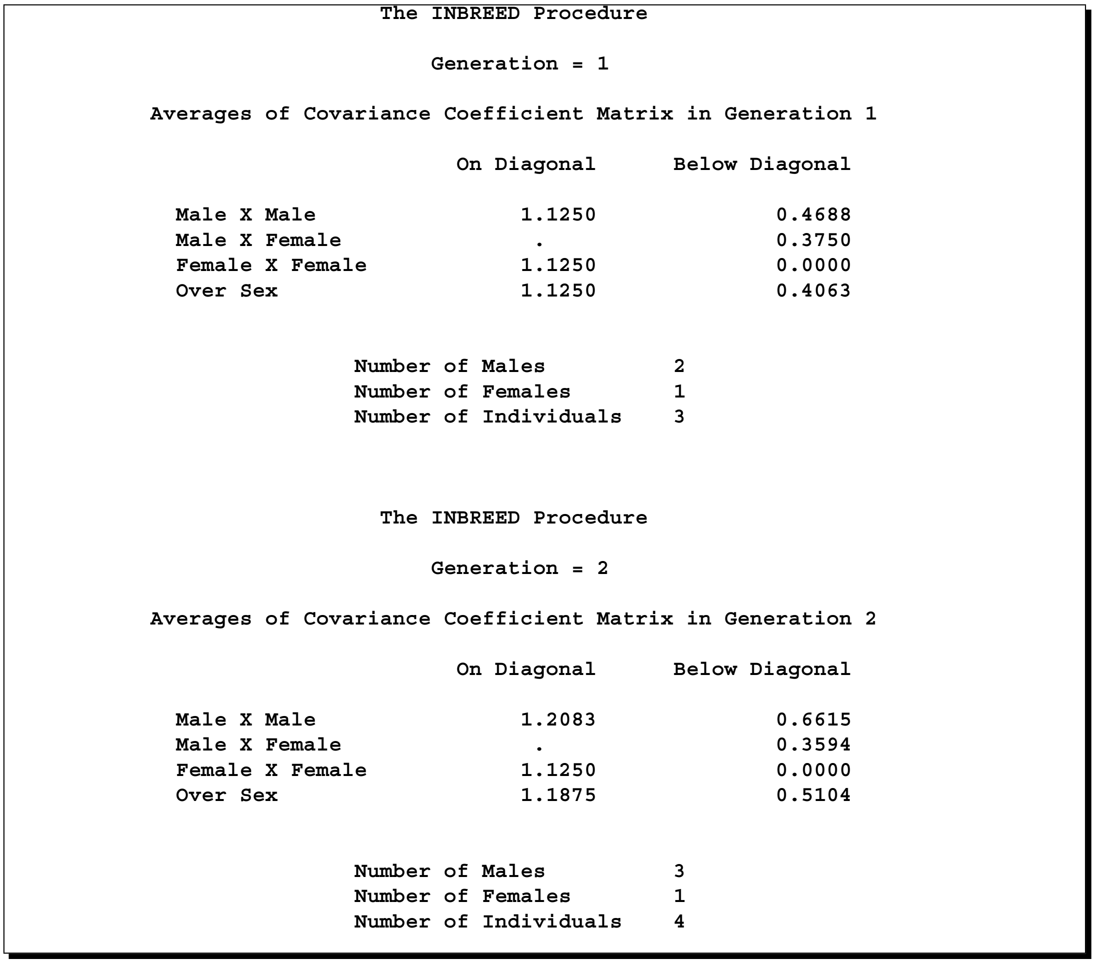


```
                              The INBREED Procedure

                                 Generation = 1

                             Covariance Coefficients

   Individual     Parent1     Parent2          MARK       KELLY        MIKE

   MARK           GEORGE      LISA           1.1250      0.5000      0.4688
   KELLY          SCOTT       LISA           0.5000      1.1250      0.2500
   MIKE           GEORGE      AMY            0.4688      0.2500      1.1250


                            Number of Individuals    3



                              The INBREED Procedure

                                 Generation = 2

                             Covariance Coefficients

Individual    Parent1    Parent2        DAVID       MERLE         JIM        MARK

DAVID         MARK       KELLY         1.2500      0.3047      0.8125      0.5859
MERLE         MIKE       JANE          0.3047      1.1250      0.3047      0.4688
JIM           MARK       KELLY         0.8125      0.3047      1.2500      0.5859
MARK          MIKE       KELLY         0.5859      0.4688      0.5859      1.1250


                            Number of Individuals    4
```

Figure 7.2. Analysis for a Nonoverlapping Population

You may also want to see covariance coefficient averages within sex categories. This is accomplished by indicating the variable defining the gender of individuals in a GENDER statement and by adding the AVERAGE option to the PROC INBREED statement. For example, the following statements produce the covariance coefficient averages shown in Figure 7.3.

```
proc inbreed data=Population covar average init=0.25;
   class Generation;
   gender Sex;
run;
```



```
                          The INBREED Procedure

                              Generation = 1

          Averages of Covariance Coefficient Matrix in Generation 1

                              On Diagonal      Below Diagonal

            Male X Male            1.1250              0.4688
            Male X Female               .              0.3750
            Female X Female        1.1250              0.0000
            Over Sex               1.1250              0.4063


                          Number of Males          2
                          Number of Females        1
                          Number of Individuals    3


                          The INBREED Procedure

                              Generation = 2

          Averages of Covariance Coefficient Matrix in Generation 2

                              On Diagonal      Below Diagonal

            Male X Male            1.2083              0.6615
            Male X Female               .              0.3594
            Female X Female        1.1250              0.0000
            Over Sex               1.1875              0.5104


                          Number of Males          3
                          Number of Females        1
                          Number of Individuals    4
```

Figure 7.3. Averages within Sex Categories for a Nonoverlapping Generation

Syntax

The following statements are available in PROC INBREED.

PROC INBREED < *options* > ;
  BY *variables* ;
  CLASS *variable* ;
  GENDER *variable* ;
  MATINGS *individual-list1 / mate-list* <, . . . > ;
  VAR *variables* ;

The PROC INBREED statement is required. Items within angle brackets (< >) are optional. The syntax of each statement is described in the following sections.

PROC INBREED Statement

PROC INBREED < *options* > ;

You can specify the following options in the PROC INBREED statement.

AVERAGE
A

produces a table of averages of coefficients for each pedigree of offspring. The AVERAGE option is used together with the GENDER statement to average the inbreeding/covariance coefficients within sex categories.

COVAR
C

specifies that all coefficients output consist of covariance coefficients rather than inbreeding coefficients.

DATA=*SAS-data-set*

names the SAS data set to be used by PROC INBREED. If you omit the DATA= option, the most recently created SAS data set is used.

IND
I

displays the individuals' inbreeding coefficients (diagonal of the inbreeding coefficients matrix) for each pedigree of offspring. If you also specify the COVAR option, the individuals' covariance coefficients (diagonal of the covariance coefficients matrix) are displayed.

INDL

displays individuals' coefficients for only the last generation of a multiparous population.

INIT=*cov*

specifies the covariance value *cov* if any of the parents are unknown; a value of 0 is assumed if you do not specify the INIT= option.

MATRIX
M

displays the inbreeding coefficient matrix for each pedigree of offspring. If you also specify the COVAR option, the covariance matrices are displayed instead of inbreeding coefficients matrices.

MATRIXL

displays coefficients for only the last generation of a multiparous population.

NOPRINT

suppresses the display of all output. Note that this option temporarily disables the Output Delivery System (ODS).

OUTCOV=*SAS-data-set*

names an output data set to contain the inbreeding coefficients. When the COVAR option is also specified, covariance estimates are output to the OUTCOV= data set instead of inbreeding coefficients.

BY Statement

BY *variables* **;**

You can specify a BY statement with PROC INBREED to obtain separate analyses on observations in groups defined by the BY variables. When a BY statement appears, the procedure expects the input DATA= data set to be sorted in order of the BY variables.

If your input data set is not sorted in ascending order, use one of the following alternatives:

- Use the SORT procedure with a similar BY statement to sort the data.
- Use the BY statement options NOTSORTED or DESCENDING in the BY statement for the INBREED procedure. As a cautionary note, the NOTSORTED option does not mean that the data are unsorted but rather that the data are arranged in groups (according to values of the BY variables), and these groups are not necessarily in alphabetical or increasing numeric order.
- Use the DATASETS procedure (in base SAS software) to create an index on the BY variables.

For more information on the BY statement, see the discussion in *SAS Language Reference: Concepts*.

CLASS Statement

CLASS *variable* **;**

To analyze the population within nonoverlapping generations, you must specify the variable that identifies generations in a CLASS statement. Values of the generation variable, called *generation numbers*, must be integers, but generations are assumed to occur in the order of their input in the input data set rather than in numerical order of the generation numbers. The name of an individual needs to be unique only within its generation.

When the MATRIXL option or the INDL option is specified, each generation requires a unique generation number in order for the specified option to work correctly. If generation numbers are not unique, all the generations with a generation number that is the same as the last generation's are output.

GENDER Statement

GENDER *variable* **;**

The GENDER statement specifies a variable that indicates the sex of the individuals. Values of the sex variable must be character beginning with 'M' or 'F', for male or female. The GENDER statement is needed only when you specify the AVERAGE option to average the inbreeding/covariance coefficients within sex categories or when you want to include a gender variable in the OUTCOV= data set.

PROC INBREED makes the following assumptions regarding the gender of individuals:

- The first parent is always assumed to be the male. See the "VAR Statement" section on page 149.
- The second parent is always assumed to be the female. See the "VAR Statement" section on page 149.
- If the gender of an individual is missing or invalid, this individual is assumed to be a female unless the population is overlapping and this individual appears as the first parent in a later observation.

Any contradictions to these rules are reported in the SAS log.

MATINGS Statement

MATINGS *individual-list1 / mate-list1* $<$, . . . ,*individual-listn / mate-listn* $>$**;**

You can specify the MATINGS statement with PROC INBREED to specify selected matings of individuals. Each individual given in *individual-list* is mated with each individual given in *mate-list*. You can write multiple mating specifications if you separate them by commas or asterisks. The procedure reports the inbreeding coefficients or covariances for each pair of mates. For example, you can use the following statement to specify the mating of an individual named 'DAVID' with an individual named 'JANE':

```
matings david / jane;
```

VAR Statement

VAR *individual parent1 parent2* $<$ *covariance* $>$ **;**

The VAR statement specifies three or four variables: the first variable contains an individual's name, the second variable contains the name of the individual's first parent, and the third variable contains the name of the individual's second parent. An optional fourth variable assigns a known value to the covariance of the individual's first and second parents in the current generation.

The first three variables in the VAR statement can be either numeric or character; however, only the first 12 characters of a character variable are recognized by the procedure. The fourth variable, if specified, must be numeric.

If you omit the VAR statement, then the procedure uses the first three unaddressed variables as the names of the individual and its parents. (Unaddressed variables are those that are not referenced in any other PROC INBREED statement.) If the input data set contains an unaddressed fourth variable, then it becomes the covariance variable.

Details

Missing Values

A missing value for a parent implies that the parent is unknown. Unknown parents are assumed to be unrelated and not inbred unless you specify the INIT= option (see the INIT= option on page 147).

When the value of the variable identifying the individual is missing, the observation is not added to the list of individuals. However, for a multiparous population, an observation with a missing individual is valid and is used for assigning covariances.

Missing covariance values are determined from the INIT=*cov* option, if specified. Observations with missing generation variables are excluded.

If the gender of an individual is missing, it is determined from the order in which it is listed on the first observation defining its progeny for an overlapping population. If it appears as the first parent, it is set to ‘M’; otherwise, it is set to ‘F’. When the gender of an individual cannot be determined, it is assigned a default value of ‘F’.

DATA= Data Set

Each observation in the input data set should contain necessary information such as the identification of an individual and the first and second parents of an individual. In addition, if a CLASS statement is specified, each observation should contain the generation identification; and, if a GENDER statement is specified, each observation should contain the gender of an individual. Optionally, each observation may also contain the covariance between the first and the second parents. Depending on how many statements are specified with the procedure, there should be enough variables in the input data set containing this information.

If you omit the VAR statement, then the procedure uses the first three *unaddressed variables* in the input data set as the names of the individual and his or her parents. Unaddressed variables in the input data set are those variables that are not referenced by the procedure in any other statements, such as CLASS, GENDER, or BY statements. If the input data set contains an unaddressed fourth variable, then the procedure uses it as the covariance variable.

If the individuals given by the variables associated with the first and second parents are not in the population, they are added to the population. However, if they are in the population, they must be defined prior to the observation that gives their progeny.

When there is a CLASS statement, the functions of defining new individuals and assigning covariances must be separated. This is necessary because the parents of any given individual are defined in the previous generation, while covariances are assigned between individuals in the current generation.

Therefore, there could be two types of observations for a multiparous population:

- one to define new individuals in the current generation whose parents have been defined in the previous generation, as in the following, where the missing value is for the covariance variable:

```
MARK   GEORGE LISA    .    M  1
KELLY  SCOTT  LISA    .    F  1
```

- one to assign covariances between two individuals in the current generation, as in the following, where the individual's name is missing, 'MARK' and 'KELLY' are in the current generation, and the covariance coefficient between these two individuals is 0.50:

```
.      MARK   KELLY  0.50  .  1
```

Note that the observations defining individuals must precede the observation assigning a covariance value between them. For example, if a covariance is to be assigned between 'MARK' and 'KELLY', then both of them should be defined prior to the assignment observation.

Computational Details

This section describes the rules that the INBREED procedure uses to compute the covariance and inbreeding coefficients. Each computational rule is explained by an example referring to the fictitious population introduced in the "Getting Started" section on page 141.

Coancestry (or Kinship Coefficient)

To calculate the inbreeding coefficient and the covariance coefficients, use the degree of relationship by descent between the two parents, which is called *coancestry* or *kinship coefficient* (Falconer and Mackay 1996, p.85), or *coefficient of parentage* (Kempthorne 1957, p.73). Denote the coancestry between individuals X and Y by f_{XY}. For information on how to calculate the coancestries among a population, see the section "Calculation of Coancestry."

Covariance Coefficient (or Coefficient of Relationship)

The covariance coefficient between individuals X and Y is defined by

$$\mathrm{Cov(X,Y)} = 2f_{\mathrm{XY}}$$

where f_{XY} is the coancestry between X and Y. The covariance coefficient is sometimes called the *coefficient of relationship* or the *theoretical correlation* (Falconer and Mackay 1996, p.153; Crow and Kimura 1970, p.134). If a covariance coefficient cannot be calculated from the individuals in the population, it is assigned to an initial value. The initial value is set to 0 if the INIT= option is not specified or to *cov* if INIT=*cov*. Therefore, the corresponding initial coancestry is set to 0 if the INIT= option is not specified or to $\frac{1}{2}cov$ if INIT=*cov*.

Inbreeding Coefficients

The inbreeding coefficient of an individual is the probability that the pair of alleles carried by the gametes that produced it are identical by descent (Falconer and Mackay 1996, Chapter 5; Kempthorne 1957, Chapter 5). For individual X, denote its inbreeding coefficient by F_{X}. The inbreeding coefficient of an individual is equal to the coancestry between its parents. For example, if X has parents A and B, then the inbreeding coefficient of X is

$$F_{\mathrm{X}} = f_{\mathrm{AB}}$$

Calculation of Coancestry

Given individuals X and Y, assume that X has parents A and B and that Y has parents C and D. For nonoverlapping generations, the basic rule to calculate the coancestry between X and Y is given by the following formula (Falconer and Mackay 1996, p.86):

$$f_{\mathrm{XY}} = \frac{1}{4}\left(f_{\mathrm{AC}} + f_{\mathrm{AD}} + f_{\mathrm{BC}} + f_{\mathrm{BD}}\right)$$

And the inbreeding coefficient for an offspring of X and Y, called Z, is the coancestry between X and Y:

$$F_{\mathrm{Z}} = f_{\mathrm{XY}}$$

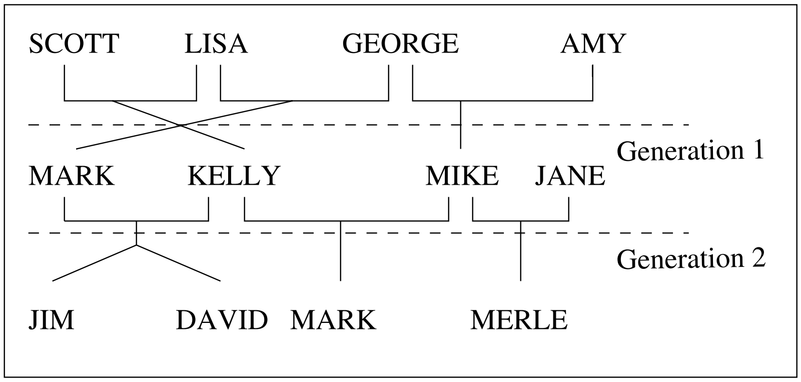


Figure 7.4. Inbreeding Relationship for Nonoverlapping Population

For example, in Figure 7.4, 'JIM' and 'MARK' from Generation 2 are progenies of 'MARK' and 'KELLY' and of 'MIKE' and 'KELLY' from Generation 1, respectively. The coancestry between 'JIM' and 'MARK' is

$$f_{\text{JIM,MARK}} = \frac{1}{4}\left(f_{\text{MARK,MIKE}} + f_{\text{MARK, KELLY}} + f_{\text{KELLY, MIKE}} + f_{\text{KELLY, KELLY}}\right)$$

From the covariance matrix for Generation=1 in Figure 7.2 (page 145) and the relationship that coancestry is half of the covariance coefficient,

$$f_{\text{JIM, MARK}} = \frac{1}{4}\left(\frac{0.4688}{2} + \frac{0.5}{2} + \frac{0.25}{2} + \frac{1.125}{2}\right) = 0.29298$$

For overlapping generations, if X is older than Y, then the basic rule (on page 152) can be simplified to

$$F_{\text{Z}} = f_{\text{XY}} = \frac{1}{2}\left(f_{\text{XC}} + f_{\text{XD}}\right)$$

That is, the coancestry between X and Y is the average of coancestries between older X with younger Y's parents. For example, in Figure 7.5, the coancestry between 'KELLY' and 'DAVID' is

$$f_{\text{KELLY,DAVID}} = \frac{1}{2}\left(f_{\text{KELLY,MARK}} + f_{\text{KELLY, KELLY}}\right)$$

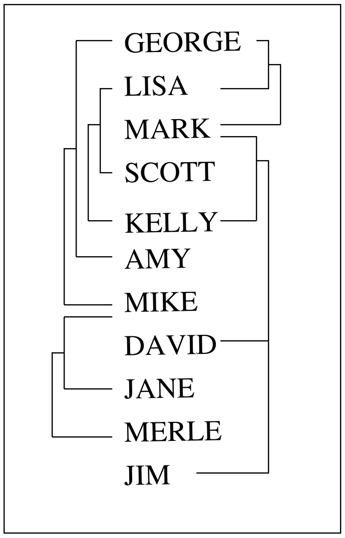


Figure 7.5. Inbreeding Relationship for Overlapping Population

This is so because 'KELLY' is defined before 'DAVID'; therefore, 'KELLY' is not younger than 'DAVID', and the parents of 'DAVID' are 'MARK' and 'KELLY'. The covariance coefficient values Cov(KELLY,MARK) and Cov(KELLY,KELLY) from the matrix in Figure 7.1 on page 144 yield that the coancestry between ‘KELLY’ and ‘DAVID’ is

$$f_{\text{KELLY, DAVID}} = \frac{1}{2}\left(\frac{0.5}{2} + \frac{1.125}{2}\right) = 0.40625$$

The numerical values for some initial coancestries must be known in order to use these rule. Either the parents of the first generation have to be unrelated, with $f = 0$ if the INIT= option is not specified in the PROC statement, or their coancestries must have an initial value of $\frac{1}{2}cov$, where *cov* is set by the INIT= option. Then the subsequent coancestries among their progenies and the inbreeding coefficients of their progenies in the rest of the generations are calculated using these initial values.

Special rules need to be considered in the calculations of coancestries for the following cases.

Self-Mating

The coancestry for an individual X with itself, f_{XX}, is the inbreeding coefficient of a progeny that is produced by self-mating. The relationship between the inbreeding coefficient and the coancestry for self-mating is

$$f_{\text{XX}} = \frac{1}{2}\left(1 + F_{\text{X}}\right)$$

The inbreeding coefficient F_{X} can be replaced by the coancestry between X’s parents A and B, f_{AB}, if A and B are in the population:

$$f_{\text{XX}} = \frac{1}{2}\left(1 + f_{\text{AB}}\right)$$

If X’s parents are not in the population, then F_{X} is replaced by the initial value $\frac{1}{2}cov$ if *cov* is set by the INIT= option, or F_{X} is replaced by 0 if the INIT= option is not specified. For example, the coancestry of ‘JIM’ with himself is

$$f_{\text{JIM,JIM}} = \frac{1}{2}\left(1 + f_{\text{MARK, KELLY}}\right)$$

where ‘MARK’ and ‘KELLY’ are the parents of ‘JIM’. Since the covariance coefficient Cov(MARK,KELLY) is 0.5 in Figure 7.1 on page 144 and also in the covariance matrix for GENDER=1 in Figure 7.2 on page 145, the coancestry of ‘JIM’ with himself is

$$f_{\text{JIM,JIM}} = \frac{1}{2}\left(1 + \frac{0.5}{2}\right) = 0.625$$

When INIT=0.25, then the coancestry of ‘JANE’ with herself is

$$f_{\text{JANE,JANE}} = \frac{1}{2}\left(1 + \frac{0.25}{2}\right) = 0.5625$$

because ‘JANE’ is not an offspring in the population.

Offspring and Parent Mating

Assuming that X's parents are A and B, the coancestry between X and A is

$$f_{\mathrm{XA}} = \frac{1}{2}\left(f_{\mathrm{AB}} + f_{\mathrm{AA}}\right)$$

The inbreeding coefficient for an offspring of X and A, denoted by Z, is

$$F_{\mathrm{Z}} = f_{\mathrm{XA}} = \frac{1}{2}\left(f_{\mathrm{AB}} + f_{\mathrm{AA}}\right)$$

For example, 'MARK' is an offspring of 'GEORGE' and 'LISA', so the coancestry between 'MARK' and 'LISA' is

$$f_{\mathrm{MARK,\ LISA}} = \frac{1}{2}\left(f_{\mathrm{LISA,GEORGE}} + f_{\mathrm{LISA,\ LISA}}\right)$$

From the covariance coefficient matrix in Figure 7.1 on page 144, $f_{\mathrm{LISA,GEORGE}} = 0.25/2 = 0.125$, $f_{\mathrm{LISA,LISA}} = 1.125/2 = 0.5625$, so that

$$f_{\mathrm{MARK,\ LISA}} = \frac{1}{2}(0.125 + 0.5625) = 0.34375$$

Thus, the inbreeding coefficient for an offspring of 'MARK' and 'LISA' is 0.34375.

Full Sibs Mating

This is a special case for the basic rule given at the beginning of the section "Calculation of Coancestry" on page 152. If X and Y are full sibs with same parents A and B, then the coancestry between X and Y is

$$f_{\mathrm{XY}} = \frac{1}{4}\left(2f_{\mathrm{AB}} + f_{\mathrm{AA}} + f_{\mathrm{BB}}\right)$$

and the inbreeding coefficient for an offspring of A and B, denoted by Z, is

$$F_{\mathrm{Z}} = f_{\mathrm{XY}} = \frac{1}{4}\left(2f_{\mathrm{AB}} + f_{\mathrm{AA}} + f_{\mathrm{BB}}\right)$$

For example, 'DAVID' and 'JIM' are full sibs with parents 'MARK' and 'KELLY', so the coancestry between 'DAVID' and 'JIM' is

$$f_{\mathrm{DAVID,\ JIM}} = \frac{1}{4}\left(2f_{\mathrm{MARK,KELLY}} + f_{\mathrm{MARK,\ MARK}} + f_{\mathrm{KELLY,\ KELLY}}\right)$$

Since the coancestry is half of the covariance coefficient, from the covariance matrix in Figure 7.1 on page 144,

$$f_{\mathrm{DAVID,JIM}} = \frac{1}{4}\left(2 \times \frac{0.5}{2} + \frac{1.125}{2} + \frac{1.125}{2}\right) = 0.40625$$

Unknown or Missing Parents

When individuals or their parents are unknown in the population, their coancestries are assigned by the value $\frac{1}{2}cov$ if *cov* is set by the INIT= option or by the value 0 if the INIT= option is not specified. That is, if either A or B is unknown, then

$$f_{\mathrm{AB}} = \frac{1}{2}cov$$

For example, 'JANE' is not in the population, and since 'JANE' is assumed to be defined just before the observation at which 'JANE' appears as a parent (that is, between observations 4 and 5), then 'JANE' is not older than 'SCOTT'. The coancestry between 'JANE' and 'SCOTT' is then obtained by using the simplified basic rule (see page 153):

$$f_{\mathrm{SCOTT,JANE}} = \frac{1}{2}\left(f_{\mathrm{SCOTT},\cdot} + f_{\mathrm{SCOTT},\cdot}\right)$$

Here, dots $(\cdot)$ indicate JANE's unknown parents. Therefore, $f_{\mathrm{SCOTT},\cdot}$ is replaced by $\frac{1}{2}cov$, where *cov* is set by the INIT= option. If INIT=0.25, then

$$f_{\mathrm{SCOTT,JANE}} = \frac{1}{2}\left(\frac{0.25}{2} + \frac{0.25}{2}\right) = 0.125$$

For a more detailed discussion on the calculation of coancestries, inbreeding coefficients, and covariance coefficients, refer to Falconer and Mackay (1996), Kempthorne (1957), and Crow and Kimura (1970).

OUTCOV= Data Set

The OUTCOV= data set has the following variables:

- a list of BY variables, if there is a BY statement
- the generation variable, if there is a CLASS statement
- the gender variable, if there is a GENDER statement
- _Type_, a variable indicating the type of observation. The valid values of the _Type_ variable are 'COV' for covariance estimates and 'INBREED' for inbreeding coefficients.
- _Panel_, a variable indicating the panel number used when populations delimited by BY groups contain different numbers of individuals. If there are n individuals in the first BY group and if any subsequent BY group contains a larger population, then its covariance/inbreeding matrix is divided into panels, with each panel containing n columns of data. If you put these panels side by side in increasing _Panel_ number order, then you can reconstruct the covariance or inbreeding matrix.

- _Col_, a variable used to name columns of the inbreeding or covariance matrix. The values of this variable start with ‘COL’, followed by a number indicating the column number. The names of the individuals corresponding to any given column i can be found by reading the individual’s name across the row that has a _Col_ value of ‘COLi’. When the inbreeding or covariance matrix is divided into panels, all the rows repeat for the first n columns, all the rows repeat for the next n columns, and so on.
- the variable containing the names of the individuals, that is, the first variable listed in the VAR statement
- the variable containing the names of the first parents, that is, the second variable listed in the VAR statement
- the variable containing the names of the second parents, that is, the third variable listed in the VAR statement
- a list of covariance variables Col1-Col$n$, where n is the maximum number of individuals in the first population

The functions of the variables _Panel_ and _Col_ can best be demonstrated by an example. Assume that there are three individuals in the first BY group and that, in the current BY group (Byvar=2), there are five individuals with the following covariance matrix.

COV	**1**	**2**	**3**	**4**	**5**
1	Cov(1,1)	Cov(1,2)	Cov(1,3)	Cov(1,4)	Cov(1,5)
2	Cov(2,1)	Cov(2,2)	Cov(2,3)	Cov(2,4)	Cov(2,5)
3	Cov(3,1)	Cov(3,2)	Cov(3,3)	Cov(3,4)	Cov(3,5)
4	Cov(4,1)	Cov(4,2)	Cov(4,3)	Cov(4,4)	Cov(4,5)
5	Cov(5,1)	Cov(5,2)	Cov(5,3)	Cov(5,4)	Cov(5,5)
	Panel 1			Panel 2	

Then the OUTCOV= data set appears as follows.

Byvar	_Panel_	_Col_	Individual	Parent	Parent2	Col1	Col2	Col3
2	1	COL1	1			Cov(1,1)	Cov(1,2)	Cov(1,3)
2	1	COL2	2			Cov(2,1)	Cov(2,2)	Cov(2,3)
2	1	COL3	3			Cov(3,1)	Cov(3,2)	Cov(3,3)
2	1		4			Cov(4,1)	Cov(4,2)	Cov(4,3)
2	1		5			Cov(5,1)	Cov(5,2)	Cov(5,3)
2	2		1			Cov(1,4)	Cov(1,5)	.
2	2		2			Cov(2,4)	Cov(2,5)	.
2	2		3			Cov(3,4)	Cov(3,5)	.
2	2	COL1	4			Cov(4,4)	Cov(4,5)	.
2	2	COL2	5			Cov(5,4)	Cov(5,5)	.

Notice that the first three columns go to the first panel (_Panel_=1), and the remaining two go to the second panel (_Panel_=2). Therefore, in the first panel, 'COL1', 'COL2', and 'COL3' correspond to individuals 1, 2, and 3, respectively, while in the second panel, 'COL1' and 'COL2' correspond to individuals 4 and 5, respectively.

Displayed Output

The INBREED procedure can output either covariance coefficients or inbreeding coefficients. Note that the following items can be produced for each generation if generations do not overlap.

The output produced by PROC INBREED can be any or all of the following items:

- a matrix of coefficients
- coefficients of the individuals
- coefficients for selected matings

ODS Table Names

PROC INBREED assigns a name to each table it creates. You can use these names to reference the table when using the Output Delivery System (ODS) to select tables and create output data sets. These names are listed in the following table.

Table 7.1. ODS Tables Produced in PROC INBREED

ODS Table Name	Description	Statement	Option
AvgCovCoef	Averages of covariance coefficient matrix	GENDER	COVAR and AVERAGE
AvgInbreedingCoef	Averages of inbreeding coefficient matrix	GENDER	AVERAGE
CovarianceCoefficient	Covariance coefficient table	PROC	COVAR and MATRIX
InbreedingCoefficient	Inbreeding coefficient table	PROC	MATRIX
IndividualCovCoef	Covariance coefficients of individuals	PROC	IND and COVAR
IndividualInbreedingCoef	Inbreeding coefficients of individuals	PROC	IND
MatingCovCoef	Covariance coefficients of matings	MATINGS	COVAR
MatingInbreedingCoef	Inbreeding coefficients of matings	MATINGS	
NumberOfObservations	Number of observations	PROC	

Examples

Example 7.1. Monoecious Population Analysis

The following example shows a covariance analysis within nonoverlapping generations for a monoecious population. Parents of generation 1 are unknown and therefore assumed to be unrelated. The result appears in Output 7.1.1.

```
data Monoecious;
   input Generation Individual Parent1 Parent2 Covariance @@;
   datalines;
1 1 . .   .      1 2 . .   .       1 3 . .   .
2 1 1 1   .      2 2 1 2   .       2 3 2 3   .
3 1 1 2   .      3 2 1 3   .       3 3 2 1   .
3 4 1 3   .      3 . 2 3 0.50      3 . 4 3 1.135
;

title 'Inbreeding within Nonoverlapping Generations';
proc inbreed ind covar matrix data=Monoecious;
   class Generation;
run;
```

Output 7.1.1. Monoecious Population Analysis

```
                 Inbreeding within Nonoverlapping Generations

                            The INBREED Procedure

                               Generation = 1

                           Covariance Coefficients

     Individual    Parent1    Parent2          1           2           3

     1                                    1.0000           .           .
     2                                         .      1.0000           .
     3                                         .           .      1.0000


                 Inbreeding within Nonoverlapping Generations

                            The INBREED Procedure

                               Generation = 1

                    Covariance Coefficients of Individuals

               Individual    Parent1    Parent2     Coefficient

               1                                         1.0000
               2                                         1.0000
               3                                         1.0000


                         Number of Individuals    3
```

```
            Inbreeding within Nonoverlapping Generations

                       The INBREED Procedure

                           Generation = 2

                      Covariance Coefficients

Individual    Parent1    Parent2           1           2           3

1             1          1            1.5000      0.5000       .
2             1          2            0.5000      1.0000      0.2500
3             2          3             .          0.2500      1.0000


            Inbreeding within Nonoverlapping Generations

                       The INBREED Procedure

                           Generation = 2

                  Covariance Coefficients of Individuals

          Individual    Parent1    Parent2     Coefficient

          1             1          1                1.5000
          2             1          2                1.0000
          3             2          3                1.0000


                     Number of Individuals    3
```

```
                 Inbreeding within Nonoverlapping Generations

                           The INBREED Procedure

                              Generation = 3

                          Covariance Coefficients

Individual    Parent1    Parent2           1          2          3          4

1             1          2            1.2500     0.5625     0.8750     0.5625
2             1          3            0.5625     1.0000     1.1349     0.6250
3             2          1            0.8750     1.1349     1.2500     1.1349
4             1          3            0.5625     0.6250     1.1349     1.0000


                 Inbreeding within Nonoverlapping Generations

                           The INBREED Procedure

                              Generation = 3

                    Covariance Coefficients of Individuals

                Individual    Parent1    Parent2     Coefficient

                1             1          2                1.2500
                2             1          3                1.0000
                3             2          1                1.2500
                4             1          3                1.0000


                          Number of Individuals    4
```

Note that, since the parents of the first generation are unknown, off-diagonal elements of the covariance matrix are all 0s and on-diagonal elements are all 1s. If there is an INIT=*cov* value, then the off-diagonal elements would be equal to *cov*, while on-diagonal elements would be equal to $1 + cov/2$.

In the third generation, individuals 2 and 4 are full siblings, so they belong to the same family. Since PROC INBREED computes covariance coefficients between families, the second and fourth columns of inbreeding coefficients are the same, except that their intersections with the second and fourth rows are reordered. Notice that, even though there is an observation to assign a covariance of 0.50 between individuals 2 and 3 in the third generation, the covariance between 2 and 3 is set to 1.135, the same value assigned between 4 and 3. This is because families get the same covariances, and later specifications override previous ones.

Example 7.2. Pedigree Analysis

In the following example, an inbreeding analysis is performed for a complicated pedigree. This analysis includes computing selective matings of some individuals and inbreeding coefficients of all individuals. Also, inbreeding coefficients are averaged within sex categories. The result appears in Output 7.2.1.

```
data Swine;
   input Swine_Number $ Sire $ Dam $ Sex $;
   datalines;
3504 2200 2501  M
3514 2521 3112  F
3519 2521 2501  F
2501 2200 3112  M
2789 3504 3514  F
3501 2521 3514  M
3712 3504 3514  F
3121 2200 3501  F
;

title 'Least Related Matings';
proc inbreed data=Swine ind average;
   var Swine_Number Sire Dam;
   matings 2501 / 3501 3504 ,
           3712 / 3121;
   gender Sex;
run;
```

Note the following from Output 7.2.1:

- Observation 4, which defines Swine_Number=2501, should precede the first and third observations where the progeny for 2501 are given. PROC INBREED ignores observation 4 since it is given out of order. As a result, the parents of 2501 are missing or unknown.
- The first column in the "Inbreeding Averages" table corresponds to the averages taken over the on-diagonal elements of the inbreeding coefficients matrix, and the second column gives averages over the off-diagonal elements.

Output 7.2.1. Pedigree Analysis

```
               Least Related Matings

               The INBREED Procedure

       Inbreeding Coefficients of Individuals

  Swine_
  Number      Sire         Dam          Coefficient

  2200                                        .
  2501                                        .
  3504        2200         2501               .
  2521                                        .
  3112                                        .
  3514        2521         3112               .
  3519        2521         2501               .
  2789        3504         3514               .
  3501        2521         3514             0.2500
  3712        3504         3514               .
  3121        2200         3501               .


               Least Related Matings

               The INBREED Procedure

         Inbreeding Coefficients of Matings

        Sire        Dam          Coefficient

        2501        3501               .
        2501        3504             0.2500
        3712        3121             0.1563


     Averages of Inbreeding Coefficient Matrix

                       Inbreeding        Coancestry

Male X Male                0.0625            0.1042
Male X Female               .                0.1362
Female X Female            0.0000            0.1324
Over Sex                   0.0227            0.1313


            Number of Males           4
            Number of Females         7
            Number of Individuals    11
```

Example 7.3. Pedigree Analysis with BY Groups

This example demonstrates the structure of the OUTCOV= data set created by PROC INBREED. Note that the first BY group has three individuals, while the second has five. Therefore, the covariance matrix for the second BY group is broken up into two panels, as shown in Output 7.3.1.

```
data Swine;
   input Group Swine_Number $ Sire $ Dam $ Sex $;
   datalines;
1  2789 3504 3514  F
```

```
2  2501 2200 3112  .
2  3504 2501 3782  M
;

proc inbreed data=Swine covar noprint outcov=Covariance
             init=0.4;
   var Swine_Number Sire Dam;
   gender Sex;
   by Group;
run;

title 'Printout of OUTCOV= data set';
proc print data=Covariance;
   format Col1-Col3 4.2;
run;
```

Output 7.3.1. Pedigree Analysis with BY Groups

```
                          Printout of OUTCOV= data set

                                             Swine_
OBS  Group  Sex  _TYPE_  _PANEL_  _COL_  Number  Sire  Dam   COL1  COL2  COL3

  1    1     M    COV       1     COL1    3504               1.20  0.40  0.80
  2    1     F    COV       1     COL2    3514               0.40  1.20  0.80
  3    1     F    COV       1     COL3    2789   3504  3514  0.80  0.80  1.20
  4    2     M    COV       1     COL1    2200               1.20  0.40  0.80
  5    2     F    COV       1     COL2    3112               0.40  1.20  0.80
  6    2     M    COV       1     COL3    2501   2200  3112  0.80  0.80  1.20
  7    2     F    COV       1             3782               0.40  0.40  0.40
  8    2     M    COV       1             3504   2501  3782  0.60  0.60  0.80
  9    2     M    COV       2             2200               0.40  0.60   .
 10    2     F    COV       2             3112               0.40  0.60   .
 11    2     M    COV       2             2501   2200  3112  0.40  0.80   .
 12    2     F    COV       2     COL1    3782               1.20  0.80   .
 13    2     M    COV       2     COL2    3504   2501  3782  0.80  1.20   .
```

References

Crow, J.F. and Kimura, M. (1970), *An Introduction to Population Genetics Theory*, New York: Harper and Row.

Falconer, D. S. and Mackay, T. F. C. (1996), *Introduction to Quantitative Genetics*, Fourth Edition, London: Longman.

Kempthorne, O. (1957), *An Introduction to Genetic Statistics*, New York: John Wiley and Sons, Inc.

Chapter 8
The PSMOOTH Procedure

Chapter Contents

Chapter 8
The PSMOOTH Procedure

Overview

In the search for complex disease genes, linkage and/or association tests are often performed on markers from a genome-wide scan or SNPs from a finely scaled map. This means hundreds or even thousands of hypotheses are being simultaneously tested. Plotting the negative log p-values of all the marker tests will reveal many peaks that indicate significant test results, some of which are false positives. In order to reduce the number of false positives or improve power, smoothing methods can be applied that take into account p-values from neighboring, and possibly correlated, markers. That is, the peak length can be used to indicate significance in addition to the peak height. The PSMOOTH procedure offers smoothing methods that implement Simes' method (1986), Fisher's method (1932), and/or the truncated product method (TPM) (2002) for multiple hypothesis testing. These methods modify the p-value from each marker test using a function of its original p-value and the p-values of the tests on the nearest markers. Since the number of hypothesis tests being performed is not reduced, adjustments to correct the smoothed p-values for multiple testing are available as well.

PROC PSMOOTH can take any data set containing any number of columns of p-values as an input data set, including the output data sets from the CASECONTROL and FAMILY procedures (see Chapter 3 and Chapter 4 for more information).

Getting Started

Example

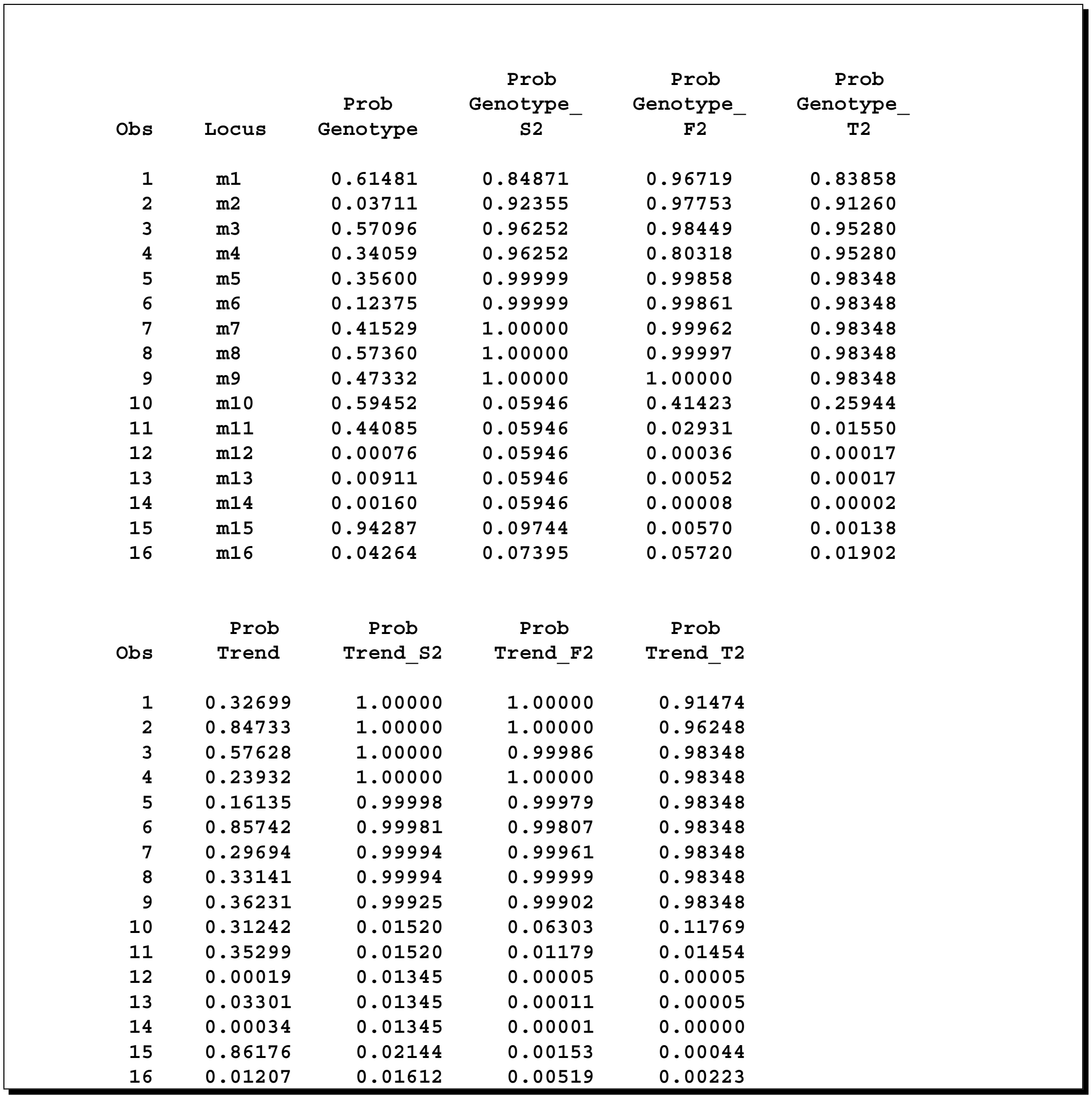
Suppose you want to test 16 markers for association with a disease using the genotype case-control and trend tests in PROC CASECONTROL. You are concerned about the multiple hypothesis testing issue, and so you also want to run PROC PSMOOTH on the output data set from PROC CASECONTROL in order to eliminate the number of false positives found using the individual p-values from the marker-trait association tests.

```
data in;
   input affected (m1-m16) ($);
   datalines;
1 1/2 2/2 2/2 2/2 1/1 2/2 1/2 1/2 1/1 1/2 1/2 2/2 2/2 2/2 2/2 1/2
1 1/2 1/1 1/2 1/2 1/1 1/1 1/2 1/1 1/2 1/2 1/1 2/2 1/1 1/2 1/1 1/2
1 1/1 2/2 1/2 1/2 1/1 1/2 1/1 1/2 1/2 2/2 2/2 1/2 1/2 1/2 2/2 1/2
1 1/1 1/2 2/2 1/2 1/2 1/1 1/2 1/2 1/2 1/1 1/1 1/2 2/2 1/2 1/1 1/1
1 1/2 1/1 1/1 1/2 2/2 1/1 1/1 1/2 1/1 2/2 1/2 2/2 2/2 2/2 1/2 1/1
1 1/2 1/1 1/2 2/2 2/2 1/1 1/1 1/2 1/2 1/2 2/2 2/2 1/1 2/2 2/2 1/1
1 1/1 1/2 1/2 1/1 1/2 1/1 1/1 1/2 2/2 1/2 2/2 2/2 1/2 2/2 2/2 1/1
1 1/2 2/2 2/2 2/2 1/2 1/2 2/2 1/2 1/2 2/2 1/2 1/2 1/1 2/2 1/2 1/2
1 1/1 2/2 1/2 1/1 1/2 2/2 1/2 1/1 1/2 2/2 2/2 2/2 1/2 2/2 2/2 1/2
1 2/2 1/2 2/2 1/1 1/2 1/1 1/1 2/2 1/2 1/1 2/2 2/2 2/2 2/2 1/2 1/1
1 1/2 1/2 2/2 2/2 1/2 2/2 1/2 1/1 1/2 1/2 1/1 1/1 2/2 1/1 1/1 1/2
1 1/1 1/1 1/2 1/2 2/2 2/2 1/2 1/2 1/1 1/2 1/1 2/2 1/1 1/1 1/2 2/2
1 1/2 1/2 1/2 1/1 2/2 1/2 1/2 2/2 1/2 1/1 1/2 1/2 1/1 1/2 1/2 1/2
1 1/2 2/2 1/1 1/2 1/2 1/2 1/2 1/1 1/1 2/2 1/2 2/2 2/2 2/2 1/1 1/2
1 1/2 1/1 2/2 1/1 2/2 1/1 1/2 1/2 1/2 1/2 1/2 2/2 1/1 2/2 1/1 1/1
1 1/1 1/1 1/1 1/2 2/2 2/2 1/2 1/1 1/2 1/2 2/2 2/2 2/2 2/2 1/1 1/1
1 1/2 1/1 1/1 1/2 1/2 1/1 2/2 1/1 1/2 1/2 2/2 2/2 1/2 2/2 1/2 1/1
1 2/2 1/1 1/2 1/1 1/2 1/1 2/2 2/2 1/1 1/2 1/2 2/2 2/2 2/2 2/2 1/2
1 2/2 2/2 2/2 1/2 1/2 2/2 2/2 2/2 2/2 1/1 1/2 2/2 1/2 1/1 1/1 1/1
1 2/2 1/1 1/2 1/2 1/2 1/1 1/2 1/2 1/2 1/2 1/2 1/2 2/2 2/2 2/2 1/2
1 1/1 2/2 1/2 1/2 1/2 1/2 1/2 1/1 1/2 1/2 2/2 1/1 2/2 2/2 1/1 1/1
1 1/2 1/2 1/2 1/1 1/1 1/2 1/1 1/1 1/1 1/1 1/2 2/2 2/2 2/2 1/1 2/2
1 1/2 2/2 2/2 2/2 1/2 2/2 1/1 1/1 1/2 1/1 1/1 2/2 2/2 1/2 2/2 2/2
1 2/2 1/2 1/2 1/2 2/2 2/2 2/2 1/2 1/2 2/2 1/2 2/2 2/2 1/2 1/2 1/1
1 2/2 2/2 1/1 1/2 2/2 2/2 1/1 1/2 1/1 1/2 1/2 1/2 2/2 2/2 1/2 1/2
0 1/1 1/2 2/2 1/2 1/1 2/2 1/2 1/2 1/2 1/2 1/1 2/2 1/2 1/1 1/2 1/2
0 1/2 1/2 2/2 1/1 1/2 1/1 2/2 2/2 1/1 2/2 1/1 1/1 1/2 1/2 1/1 1/1
0 1/2 1/2 1/1 1/1 1/2 1/2 2/2 1/2 2/2 1/1 1/2 2/2 1/1 1/1 1/1 1/1
0 1/2 1/2 2/2 1/2 1/2 1/2 1/2 2/2 1/2 1/2 2/2 1/1 2/2 1/1 2/2 2/2
0 1/2 2/2 1/1 1/1 2/2 1/2 1/2 1/2 1/2 1/1 2/2 1/1 1/2 1/1 1/2 1/2
0 1/1 1/2 1/1 2/2 1/2 2/2 2/2 2/2 1/2 2/2 1/2 2/2 2/2 1/1 1/2 1/2
0 1/1 1/2 1/2 2/2 2/2 1/2 2/2 1/1 1/2 2/2 1/2 2/2 1/2 1/1 1/1 1/2
0 1/2 1/1 2/2 1/1 1/1 1/1 2/2 2/2 1/2 1/2 2/2 1/2 1/2 1/1 2/2 2/2
0 1/1 1/2 1/2 2/2 2/2 1/2 1/1 1/2 1/2 1/2 2/2 1/1 1/2 2/2 2/2 2/2
0 2/2 2/2 1/2 1/1 1/1 2/2 1/2 1/1 2/2 2/2 1/1 1/1 2/2 1/1 1/1 2/2
0 1/2 1/2 2/2 1/2 1/2 1/2 1/2 1/2 1/2 1/2 2/2 1/1 1/2 1/1 1/1 1/2
0 1/2 1/2 1/2 1/1 2/2 2/2 1/2 2/2 1/1 1/2 1/1 2/2 1/2 1/1 1/2 1/1
0 1/2 1/2 1/2 1/2 1/2 1/2 1/2 1/2 1/1 2/2 1/2 1/1 1/1 1/1 2/2 1/2
0 1/1 2/2 2/2 1/1 1/1 1/2 2/2 1/2 1/2 1/2 1/1 1/2 1/1 1/2 1/1 2/2
0 2/2 1/2 1/2 1/2 1/2 1/2 1/2 1/1 1/1 1/1 1/1 1/1 1/2 1/1 1/1 2/2
0 1/2 1/1 1/1 1/1 1/2 1/2 1/1 1/1 1/2 1/2 1/1 1/2 1/1 1/1 2/2 1/2
0 1/1 2/2 1/1 1/2 1/1 1/1 1/1 1/2 1/1 2/2 1/1 1/1 1/2 2/2 1/2 1/2
0 1/1 1/2 1/2 1/2 1/2 2/2 1/2 1/2 2/2 1/2 1/2 1/1 1/1 1/2 1/2 2/2
0 1/2 1/2 2/2 1/1 2/2 1/1 1/2 1/1 1/2 1/2 2/2 1/2 1/2 1/2 1/2 2/2
0 2/2 1/1 1/1 1/2 1/1 2/2 1/2 1/1 1/2 2/2 1/2 1/2 1/2 2/2 2/2 1/2
0 1/1 1/2 2/2 1/1 1/1 1/1 1/2 1/2 2/2 2/2 2/2 1/1 1/2 1/2 2/2 2/2
0 1/1 1/2 1/1 1/1 1/2 1/1 1/2 2/2 1/2 2/2 1/2 1/1 2/2 2/2 1/2 1/2
0 1/2 1/2 1/2 2/2 1/2 1/2 1/2 1/1 1/2 1/2 1/2 1/1 1/1 1/2 1/2 1/2
0 1/1 1/1 1/1 1/2 1/1 1/2 1/1 1/2 2/2 1/1 1/2 2/2 1/1 1/1 2/2 1/1
0 2/2 1/1 1/2 1/1 1/2 1/2 1/2 2/2 1/1 1/2 1/1 1/1 1/1 2/2 1/1 1/2
;
```

Note that the columns marker1-marker16 contain genotypes at each of the markers, so the GENOCOL option must be used in PROC CASECONTROL to correctly read in the data.

```
proc casecontrol data=in outstat=cc_tests genotype trend genocol;
   trait affected;
   var m1-m16;
run;

proc psmooth data=cc_tests simes fisher tpm bw=2 sidak out=adj_p;
   var ProbGenotype ProbTrend;
   id Locus;
run;

proc print data=adj_p heading=h;
run;
```

This code modifies the p-values contained in the output data set from PROC CASECONTROL, first by smoothing the p-values using Simes' method, Fisher's method, and the TPM with a bandwidth of 2, then by applying Sidak's multiple testing adjustment to the smoothed p-values.



```
                                      Prob          Prob          Prob
                         Prob       Genotype_     Genotype_     Genotype_
Obs    Locus           Genotype        S2            F2            T2

  1     m1             0.61481      0.84871       0.96719       0.83858
  2     m2             0.03711      0.92355       0.97753       0.91260
  3     m3             0.57096      0.96252       0.98449       0.95280
  4     m4             0.34059      0.96252       0.80318       0.95280
  5     m5             0.35600      0.99999       0.99858       0.98348
  6     m6             0.12375      0.99999       0.99861       0.98348
  7     m7             0.41529      1.00000       0.99962       0.98348
  8     m8             0.57360      1.00000       0.99997       0.98348
  9     m9             0.47332      1.00000       1.00000       0.98348
 10     m10            0.59452      0.05946       0.41423       0.25944
 11     m11            0.44085      0.05946       0.02931       0.01550
 12     m12            0.00076      0.05946       0.00036       0.00017
 13     m13            0.00911      0.05946       0.00052       0.00017
 14     m14            0.00160      0.05946       0.00008       0.00002
 15     m15            0.94287      0.09744       0.00570       0.00138
 16     m16            0.04264      0.07395       0.05720       0.01902


          Prob         Prob          Prob          Prob
Obs      Trend       Trend_S2      Trend_F2      Trend_T2

  1     0.32699      1.00000       1.00000       0.91474
  2     0.84733      1.00000       1.00000       0.96248
  3     0.57628      1.00000       0.99986       0.98348
  4     0.23932      1.00000       1.00000       0.98348
  5     0.16135      0.99998       0.99979       0.98348
  6     0.85742      0.99981       0.99807       0.98348
  7     0.29694      0.99994       0.99961       0.98348
  8     0.33141      0.99994       0.99999       0.98348
  9     0.36231      0.99925       0.99902       0.98348
 10     0.31242      0.01520       0.06303       0.11769
 11     0.35299      0.01520       0.01179       0.01454
 12     0.00019      0.01345       0.00005       0.00005
 13     0.03301      0.01345       0.00011       0.00005
 14     0.00034      0.01345       0.00001       0.00000
 15     0.86176      0.02144       0.00153       0.00044
 16     0.01207      0.01612       0.00519       0.00223
```

Figure 8.1. PROC PSMOOTH Output Data Set

Figure 8.1 displays the original and modified p-values.

Syntax

The following statements are available in PROC PSMOOTH.

PROC PSMOOTH < *options* > **;**
BY *variables* **;**
ID *variables* **;**
VAR *variables* **;**

Items within angle brackets (< >) are optional, and statements following the PROC PSMOOTH statement can appear in any order. The VAR statement is required. The syntax of each statement is described in the following section in alphabetical order after the description of the PROC PSMOOTH statement.

PROC PSMOOTH Statement

PROC PSMOOTH < *options* > **;**

You can specify the following options in the PROC PSMOOTH statement.

BANDWIDTH=*number list*
BW=*number list*

gives the values for the bandwidths to use in combining p-values. A bandwidth of w indicates that w p-values on each side of the original p-value are included in the combining method to create a sliding window of size $2w+1$. The number list can contain any combination of the following forms, with each form separated by a comma:

$w_1, w_2, \ldots, w_n$	a list of several values
w_1 to w_2	a sequence where w_1 is the starting value, w_2 is the ending value, and the increment is 1.
w_1 to w_2 by i	a sequence where w_1 is the starting value, w_2 is the ending value, and the increment is i.

All numbers in the number list must be integers, and any negative numbers are ignored. An example of a valid number list is

```
bandwidth = 1,2, 5 to 15 by 5, 18
```

which would perform the combining of p-values using bandwidths 1, 2, 5, 10, 15, and 18, which create sliding windows of size 3, 5, 11, 21, 31, and 37, respectively.

BONFERRONI
BON

requests that the Bonferroni adjustment for multiple testing based on the number of observations in the BY group be applied to the p-values in the output data set. This adjustment is applied after the smoothing has occurred. This option is ignored if the SIDAK option is specified.

DATA=*SAS-data-set*

names the input SAS data set to be used by PROC PSMOOTH. If this option is omitted, the SAS system option _LAST_ is used, which by default is the most recently created data set.

FISHER

requests that Fisher's method for combining p-values from multiple hypotheses be applied to the original p-values.

NEGLOG

requests that all p-values, original and combined, be transformed to their negative log (base e) in the output data set; that is, for each p-value, $-\ln(p\text{-value})$ is reported in the OUT= data set. This option is useful for graphing purposes.

NEGLOG10

requests that all p-values, original and combined, be transformed to their negative log (base 10) in the output data set; that is, for each p-value, $-\log_{10}(p\text{-value})$ is reported in the OUT= data set. This option is useful for graphing purposes.

OUT=*SAS-data-set*

names the output SAS data set containing the original p-values and the new combined p-values. When this option is omitted, an output data set is created by default and named according to the DATA*n* convention.

SIDAK

requests that the Sidak adjustment for multiple testing based on the number of observations in the BY group be applied to the p-values in the output data set. This adjustment is applied after the smoothing has occurred.

SIMES

requests that Simes' method for combining p-values from multiple hypotheses be applied to the original p-values.

TAU=*number*

indicates the value of τ to be used in the TPM. The significance level for the tests can be used as the value for *number*, though this is not the only possibility. The value of *number* must be greater than 0 and less than or equal to 1. By default, *number* is set to 0.05. This option is ignored if the TPM option is not specified.

TPM

requests that the TPM for combining p-values from multiple hypotheses be applied to the original p-values.

BY Statement

BY *variables* **;**

You can specify a BY statement with PROC PSMOOTH to obtain separate analyses on observations in groups defined by the BY variables. When a BY statement appears, the procedure expects the input data set to be sorted in order of the BY variables. The *variables* are one or more variables in the input data set.

If your input data set is not sorted in ascending order, use one of the following alternatives:

- Sort the data using the SORT procedure with a similar BY statement.
- Specify the BY statement option NOTSORTED or DESCENDING in the BY statement for the PSMOOTH procedure. The NOTSORTED option does not mean that the data are unsorted but rather that the data are arranged in groups (according to values of the BY variables) and that these groups are not necessarily in alphabetical or increasing numeric order.
- Create an index on the BY variables using the DATASETS procedure (in Base SAS software).

For more information on the BY statement, refer to the discussion in *SAS Language Reference: Concepts*. For more information on the DATASETS procedure, refer to the discussion in the *SAS Procedures Guide*.

ID Statement

ID *variables* **;**

The ID statement identifies the variables from the DATA= data set that should be included in the OUT= data set.

VAR Statement

VAR *variables* **;**

The VAR statement identifies the variables containing the original p-values on which the combining methods should be performed.

Details

Statistical Computations

Methods for Smoothing p-Values

PROC PSMOOTH offers three methods for combining p-values over specified sizes of sliding windows. For each value w listed in the BANDWIDTH= option of the PROC PSMOOTH statement, a sliding window of size $2w + 1$ is used; that is, the p-values for each set of $2w + 1$ consecutive markers are considered in turn, for each value w. The approach described by Zaykin et al. (2002) is implemented, where the original p-value at the center of the sliding window is replaced by a function of the original p-value and the p-values from the w nearest markers on each side to create a new sequence of p-values. Note that for markers less than w from the beginning or end of the data set (or BY group if any variables are specified in the BY statement), the number of hypotheses tested, L, is adjusted accordingly. The three methods for combining p-values from multiple hypotheses are Simes' method, Fisher's method, and the TPM described in the following three sections. Plotting the new p-values versus the original p-values reveals the smoothing effect this technique has.

Simes' Method

Simes' method for combining p-values (1986) is performed as follows when the SIMES option is specified in the PROC PSMOOTH statement: let p_j be the original p-value at the center of the current sliding window, which contains $p_{j-w}, ..., p_{j+w}$. From these $L = 2w + 1$ p-values, the ordered p-values, $p_{(1)}, ..., p_{(L)}$ are formed. Then the new value for p_j is $\min_{1 \leq i \leq L}(Lp_{(i)}/i)$.

This method controls the type I error rate even when hypotheses are positively correlated (Sarkar and Chang 1997), which is expected for nearby markers. Thus if dependencies are suspected among tests that are performed, this method is recommended due to its conservativeness.

Fisher's Method

When the FISHER option is issued in the PROC PSMOOTH statement, Fisher's method for combining p-values (1932) is applied by replacing the p-value at the center of the current sliding window p_j by the p-value of the statistic t, where

$$t = -2 \sum_{i=j-w}^{j+w} \ln(p_i)$$

which has a χ^2_{2L} distribution under the null hypothesis of all $L = 2w + 1$ hypotheses being true.

CAUTION: t has a χ^2 distribution only under the assumption that the tests performed are mutually independent. When this assumption is violated, the probability of type I error may exceed the significance level α.

TPM

The TPM is a variation of Fisher's method that leads to a different alternative hypothesis when τ, the value specified in the TAU= option, is less than 1 (Zaykin et al. 2002). With the TPM, rejection of the null hypothesis implies there is at least one false null hypothesis among those with p-values $\leq \tau$. To calculate a combined p-value using the TPM for the p-value at the center of the sliding window, p_j, the quantity u must first be calculated as

$$u = \prod_{i=j-w}^{j+w} p_i^{I(p_i \leq \tau)}$$

Then the formula for the new value for the p-value at the center of the sliding window of L markers is

$$\sum_{k=1}^{L} \binom{L}{k} (1-\tau)^{L-k} \left(u \sum_{s=0}^{k-1} \frac{(k \log \tau - \log u)^s}{s!} I(u \leq \tau^k) + \tau^k I(u > \tau^k) \right)$$

When TAU=1 is specified, the TPM and Fisher's method are equivalent and the previous formula simplifies to

$$u \sum_{s=0}^{L-1} \frac{(-\log u)^s}{s!}$$

Multiple Testing Adjustments for p-Values

While the smoothing methods take into account the p-values from neighboring markers, the number of hypothesis tests performed does not change. Therefore, the Bonferroni and Sidak methods are offered by PROC PSMOOTH to adjust the smoothed p-values for multiple testing. The number of tests performed, R, is the number of observations in the current BY group if any variables are specified in the BY statement, or the number of observations in the entire data set if there are no variables specified in the BY statement. If both the BONFERRONI and SIDAK options are specified in the PROC PSMOOTH statement, only the Sidak method is used. Note that these adjustments will not be applied to the original column(s) of p-values; if you would like to adjust the original p-values for multiple testing, you must include a bandwidth of 0 in the BANDWIDTH= option of the PROC PSMOOTH statement.

For R tests, the p-value p is adjusted as follows according to these two methods:

Bonferroni adjustment: $\min(Rp, 1.0)$
Sidak adjustment (Sidak 1967): $1 - (1 - p)^R$

Both methods are conservative, with Sidak's slightly less conservative than Bonferroni's method.

Missing Values

Missing values in a sliding window, even at the center of the window, are simply ignored, and the number of hypotheses L is reduced accordingly. Thus the smoothing methods can be applied to any window that contains at least one nonmissing value. Any p-values in the input data set that fall outside the interval [0,1] are treated as missing.

OUT= Data Set

The output data set specified in the OUT= option of the PROC PSMOOTH statement contains any BY variables and ID variables. Then for each variable in the VAR statement, the original column is included along with a column for each method and bandwidth specified in the PROC PSMOOTH statement. These variable names are formed by adding the suffixes "_Sw", "_Fw", and "_Tw" for Simes' method, Fisher's method, and the TPM respectively and a bandwidth of size w. For example, if the options BANDWIDTH=1,4 and SIMES, FISHER, and TPM are all specified in the PROC PSMOOTH statement, and RawP is the variable specified in the VAR statement, the OUT= data set includes RawP, RawP_S1, RawP_F1, RawP_T1, RawP_S4, RawP_F4, and RawP_T4. If the NEGLOG or NEGLOG10 option is specified in the PROC PSMOOTH statement, then these columns all contain the negative logs (base e or base 10, respectively) of the p-values.

Example

Example 8.1. Displaying Plot of PROC PSMOOTH Output Data Set

Data other than the output data sets from the CASECONTROL and FAMILY procedures can be used in PROC PSMOOTH; here is an example of how to use p-values from another source.

```
data tests;
   input Marker Pvalue @@;
   datalines;
 1  0.72841     2   0.40271
 3  0.32147     4   0.91616
 5  0.27377     6   0.48943
 7  0.40131     8   0.25555
 9  0.57585    10   0.20925
11  0.01531    12   0.23306
13  0.69397    14   0.33040
15  0.97265    16   0.53639
17  0.88397    18   0.03188
19  0.13570    20   0.79138
21  0.99467    22   0.37831
23  0.86459    24   0.97092
25  0.19372    26   0.85339
27  0.32078    28   0.31806
29  0.00655    30   0.82401
31  0.65339    32   0.36115
33  0.92704    34   0.49558
35  0.64842    36   0.43606
37  0.67060    38   0.87520
39  0.78006    40   0.27252
41  0.28561    42   0.80495
43  0.98159    44   0.97030
45  0.53831    46   0.78712
47  0.88493    48   0.36260
49  0.53310    50   0.65709
51  0.26527    52   0.46860
53  0.55465    54   0.54956
55  0.44477    56   0.04933
57  0.12016    58   0.76181
59  0.80158    60   0.18244
61  0.01382    62   0.15100
63  0.04713    64   0.52655
65  0.59368    66   0.94420
67  0.60104    68   0.32848
69  0.90195    70   0.21374
71  0.95471    72   0.14145
73  0.95215    74   0.70330
75  0.19921    76   0.99086
77  0.75736    78   0.23761
79  0.87260    80   0.91472
```

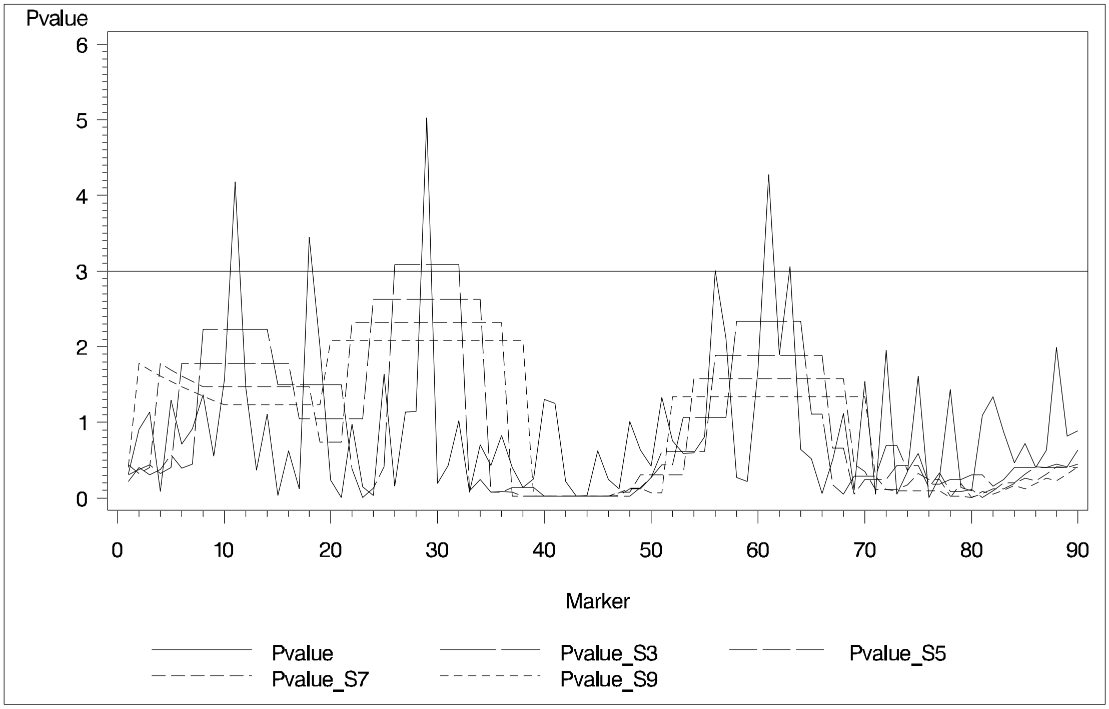
```
81  0.33650    82   0.26160
83  0.41948    84   0.62817
85  0.48721    86   0.67093
87  0.53089    88   0.13623
89  0.44344    90   0.41172
;
```

The following code will apply Simes' method for multiple hypothesis testing in order to adjust the p-values.

```
proc psmooth data=tests out=pnew simes bandwidth=3 to 9 by 2 neglog;
   var Pvalue;
   id Marker;
run;

symbol1 v=none i=join;
symbol2 v=none i=join line=5;
symbol3 v=none i=join line=4;
symbol4 v=none i=join line=3;
symbol5 v=none i=join line=2;
legend1 label=none;

proc gplot data=pnew;
   plot (Pvalue Pvalue_S3 Pvalue_S5 Pvalue_S7 Pvalue_S9)*Marker
        /overlay vref=3.0 legend=legend1;
run;
```

The NEGLOG option is used in the PROC PSMOOTH statement to facilitate plotting the p-values using the GPLOT procedure of SAS/GRAPH. The plot demonstrates the effect of the different window sizes that are implemented.

Output 8.1.1. Line Plot of Negative Log p-Values



Note how the plots become progressively smoother as the window size increases. Points above the horizontal reference line in Output 8.1.1 represent significant p-values at the 0.05 level. While six of the markers have significant p-values before adjustment, only the method using a bandwidth of 3 finds any significant markers, all in the 26–32 region. This may be an indication that the other five markers are significant only by chance; that is, they may be false positives.

References

Fisher, R.A. (1932), *Statistical Methods for Research Workers,* London: Oliver and Boyd.

Sarkar, S.K. and Chang, C-K. (1997), “The Simes Method for Multiple Hypothesis Testing with Positively Dependent Test Statistics,” *Journal of the American Statistical Association,* 92, 1601–1608.

Sidak, Z. (1967), “Rectangular Confidence Regions for the Means of Multivariate Normal Distributions,” *Journal of the American Statistical Association,* 62, 626–633.

Simes, R.J. (1986), “An Improved Bonferroni Procedure for Multiple Tests of Significance,” *Biometrika,* 73, 751–754.

Zaykin, D.V., Zhivotovsky, L.A., Westfall, P.H., and Weir, B.S. (2002), “Truncated Product Method for Combining P-values,” *Genetic Epidemiology,* 22, 170–185.

Chapter 9
The TPLOT Macro

Chapter Contents

Chapter 9
The TPLOT Macro

Overview

The %TPLOT macro creates a triangular plot that graphically displays genetic marker test results. The plot has colors and shapes representing p-value ranges for tests of the following quantities: linkage disequilibrium between pairs of markers, Hardy-Weinberg equilibrium (HWE) for individual markers, and associations between markers and a dichotomous trait (such as disease status). This is a convenient way of combining information contained in output data sets from two separate SAS/Genetics procedures and summarizing it in an easily interpretable plot. Thus, insights can be gleaned by simply studying a plot rather than by having to search through many rows of data or writing code to attempt to summarize the results.

The %TPLOT macro is a part of the SAS Autocall library, and is automatically available for use in your SAS program provided that the SAS system option MAUTOSOURCE is in effect. For more information about autocall libraries, refer to *SAS Macro Language: Reference, Version 8*, 2000.

Syntax

The %TPLOT macro has the following form:

%TPLOT *(SAS-data-set , SAS-data-set , variable [, option])*

The first argument, *SAS-data-set*, specifies the name of the SAS data set that is the output data set from the ALLELE procedure (see Chapter 2), containing the linkage disequilibrium test and HWE test p-values. A user-created data set may be used instead, but is required to contain the variables Locus1 and Locus2 and a variable ProbChi containing the p-values from the disequilibrium tests. The order in which the Locus1 and Locus2 variables are sorted is the order in which the values are displayed on the vertical and horizontal axes, respectively.

The second argument, *SAS-data-set*, specifies the name of the SAS data set that contains the p-values for the marker-trait association tests. This data set can be the output data set from the CASECONTROL procedure, the FAMILY procedure, or the PSMOOTH procedure, or it can be created by the user. A user-created data set must contain a Locus variable for the values on the axes and a variable containing p-values that is specified in the third argument, discussed in the following paragraph. The Locus variable must be in the same sorted order as the Locus1 variable in the data set named in the first argument.

The third argument, *variable*, names the variable that contains the marker-trait association p-values in the SAS data set that is specified in the second argument.

The first three arguments are required. The following option can be used with the %TPLOT macro. The option must follow the three required arguments.

ALPHA= *number*

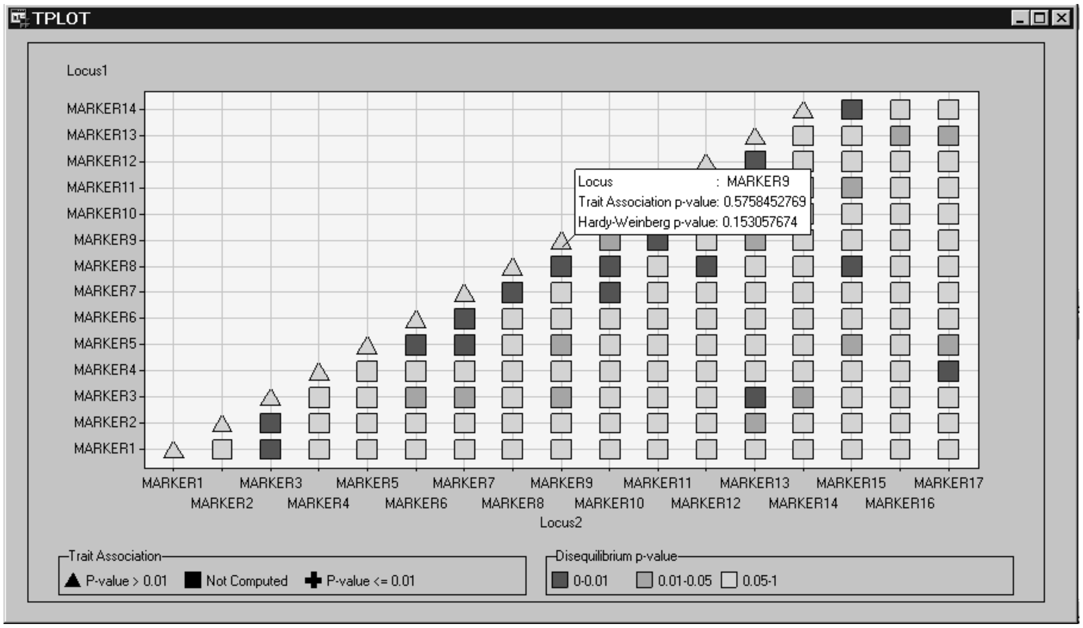
specifies the significance level for the marker-trait association test. This level is used as a cut-off for the p-value range corresponding to the symbol shape on the plot. This number must be between 0 and 1. The default is ALPHA=0.05.

Results

Plot

Running the %TPLOT macro creates a window displaying a graphical representation of the marker test results.

Here is an example of the TPLOT results window:



Locus : MARKER9
Trait Association p-value: 0.5758452769
Hardy-Weinberg p-value: 0.153057674

Figure 9.1. Results Window for TPLOT Macro

This plot contains a grid of points with symbols that represent the p-values for various marker tests. Colors and shapes of the data points are used to symbolize p-value ranges. The **Show Info About Points** button in the toolbar enables the p-values to be displayed. While holding down the left-hand mouse button on any point in the plot, the pop-up menu will display for off-diagonal points, the two markers being tested for linkage disequilibrium and the p-value of the test; it displays the marker and its p-values for the HWE test and marker-trait association test for points on the diagonal, as shown in Figure 9.1.

Disequilibrium Tests

The p-values from the linkage disequilibrium tests between all pairs of markers (or all markers within a certain range of each other) are represented by the color of the squares on the off-diagonal of the plot. For the points on the diagonal, the results from the Hardy-Weinberg equilibrium test are displayed instead of the linkage disequilibrium tests since the same marker locus is on the horizontal and vertical axes.

The three ranges of p-values that correspond to different colored symbols in the plot are

Red	[0, 0.01]
Orange	(0.01, 0.05]
Yellow	(0.05, 1]

The disequilibrium test p-values that are plotted can be provided by the output data set from PROC ALLELE, or by a user-created data set meeting the requirements described in the "Syntax" section on page 181.

Marker-Trait Association Tests

Points on the diagonal also display p-values from marker-trait association tests, using the shape of the symbol to correspond to two categories of p-values, significant and not significant. The significance level is set to 0.05 by default, but can be modified using the ALPHA= option in the %TPLOT macro. Thus, for a significance level of α, the following shapes represent the following ranges:

Plus	✚	$[0, \alpha]$
Triangle	▲	$(\alpha, 1]$

Note that the square shape ■ of the off-diagonal points does not represent a marker-trait association p-value since there are two different marker loci represented on the horizontal and vertical axes. These p-values can be provided by the output data set of PROC CASECONTROL, PROC FAMILY, or PROC PSMOOTH. Alternatively, a user-created data set that meets the conditions described in the "Syntax" section (page 181) can be used.

Menu Bar

The results window contains the following pull-down menus:

File

Close closes the results window.

Print Setup opens the printer setup utility.

Print prints the plot as it is currently shown.

Exit exits the current SAS session.

Edit

Copy copies the plot to the clipboard.

Format

Rescale Axes when selected, changes the scale of the axes to fit the entire plot in the window.

These menus are also available by clicking the right-hand mouse button anywhere in the TPLOT results window.

Toolbar

A toolbar is displayed at the top of the TPLOT results window. Use the toolbar to display information about points on the plot or to modify the plot's appearance. Tool tips are displayed when you place your mouse pointer over an icon in the toolbar.

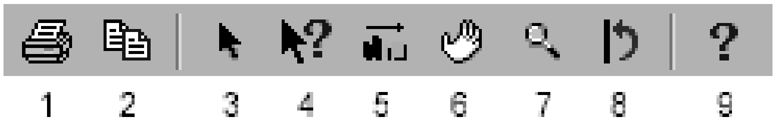


Figure 9.2. Toolbar for the %TPLOT Results Window

Tool icons from left to right are as follows:

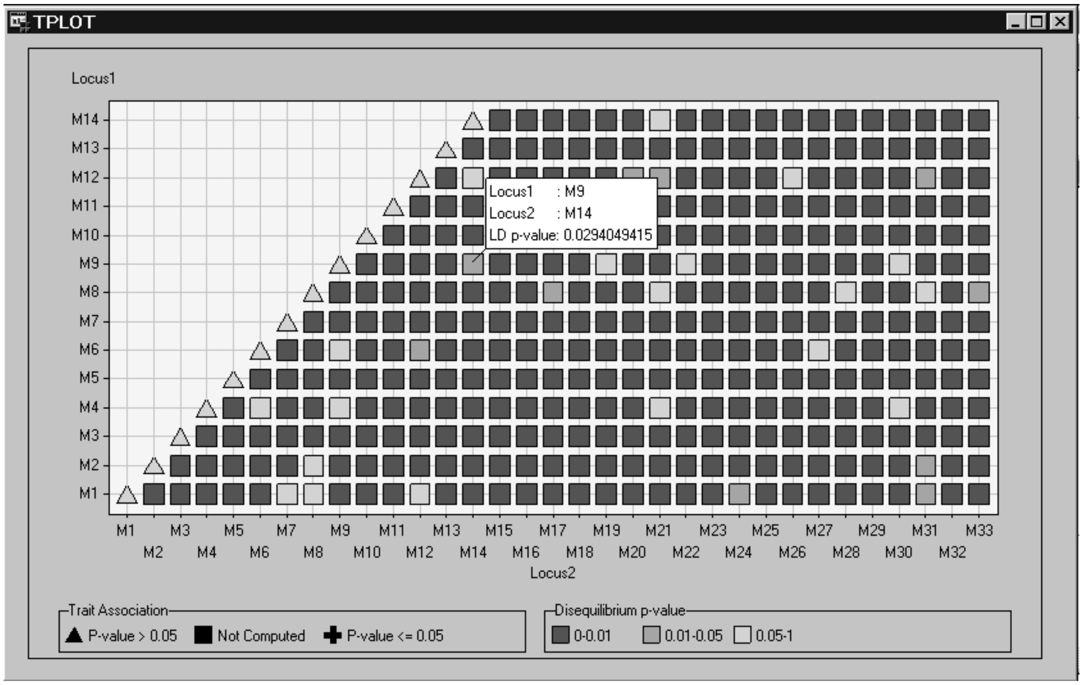
1. **Print** - prints the plot.
2. **Copy** - copies the plot to the clipboard.
3. **Select a Node or Point** - activates a point on the plot.
4. **Show Info About Points** - displays a text box with information about the selected point.
5. **Scroll Data** - scrolls across data points within the plot. Use this tool when the plot is not able to display all of the points in a single frame.
6. **Move Graph** - moves the plot within the window.
7. **Zoom In/Out** - reduces or increases the size of the plot.
8. **Reset** - returns the plot to its default settings.
9. **What's This?** - displays the help for the results window.

Example

Here is an example of the code that can be used to create the triangular plot of p-values for the data set pop22. This data set is in the proper form for a PROC ALLELE input data set, containing columns of alleles for 150 markers.

```
proc allele data=pop22 outstat=ldstats noprint maxdist=150;
   var a1-a300;
run;

proc casecontrol data=pop22 outstat=assocstats genotype;
   trait affected;
   var a1-a300;
run;

proc psmooth data=assocstats out=sm_assocstats bw=5 simes;
   id Locus;
   var ProbGenotype;
run;

%tplot(ldstats, sm_assocstats, ProbGenotype_S5);
```

Note that the output data set from PROC CASECONTROL can be used in place of the output data set from PROC PSMOOTH if you wish to use unadjusted p-values. This code creates the following plot in the TPLOT window:



Locus1 : M9
Locus2 : M14
LD p-value: 0.0294049415

Figure 9.3. Results Window for TPLOT Macro

Figure 9.3 displays the bottom left-hand corner of the plot. The pop-up window is displayed by selecting **Show Info About Points** from the toolbar then holding the left-hand mouse button over the point shown. The orange color of this point indicates that the p-value for testing that there is no linkage disequilibrium between M9 and M14 is between 0.01 and 0.05. The pop-up window provides the exact value of this p-value.

Other parts of the plot can be viewed by selecting **Scroll Data** from the toolbar. Alternatively, the entire plot can be viewed in the window by selecting **Format →Rescale Axes** from the menu bar. This creates the following view of the plot:

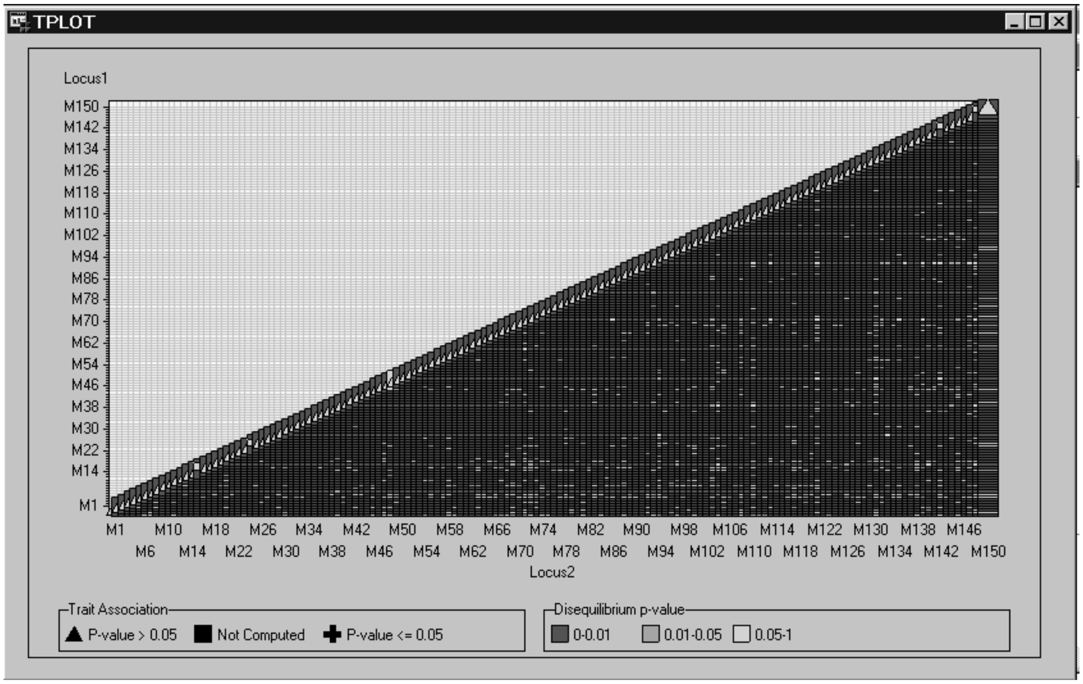


Figure 9.4. Results Window for TPLOT Macro

The view shown in Figure 9.4 displays all the data points at once.

Subject Index

Syntax Index

Your Turn

If you have comments or suggestions about *SAS/Genetics*™ *9.1 User's Guide,* please send them to us on a photocopy of this page or send us electronic mail.

For comments about this book, please return the photocopy to

SAS Publishing
SAS Campus Drive
Cary, NC 27513
E-mail: **`yourturn@sas.com`**

For suggestions about the software, please return the photocopy to

SAS Institute Inc.
Technical Support Division
SAS Campus Drive
Cary, NC 27513
E-mail: **`suggest@sas.com`**