Mixed stock analysis in R: getting started with the `mixstock` package

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1 Introduction

The `mixstock` package is a set of routines written in the R language [7] for doing mixed stock analysis using data on markers gathered from source populations and from one or more mixed populations. The package was developed for analyzing mitochondrial DNA (mtDNA) markers from sea turtle populations, but should be applicable to any case with discrete sources, discrete mixed populations, and discrete markers. (However, I do refer to sources as "rookeries" and markers as "haplotypes" throughout this document.) The package is intended to be self-contained, but some familiarity with R or S-PLUS will be helpful. (Some familiarity with your computer's operating system, which is probably Microsoft Windows, is also assumed.) The statistical methods implemented in the package are described in [1] and [6].

If you are feeling impatient and confident, turn to "Quick Start" (section 6).

2 Installation

You can skip this section if you are reading this file via the `vignette()` command in R— that means you've already successfully installed the package.

To get started, you will have to download and install the R package, a general-purpose statistics and graphics package, from `http://cran.us.`

r-project.org/bin/windows/base/ if you are in the US (or see http://www.r-project.org/mirrors.html for a list of alternative "mirror sites" closer to you). You will download a file called R-x.y.z-win32.exe which will install R for you, when executed; x.y.z stands for the current version of R 2.4.1 as of October 18, 2007).

The following installation instructions assume you are using a "modern" Microsoft Windows system (tested on 2000 and XP); it is possible to use R, and the mixstock package, on other operating systems — please contact the authors for more information. (The package has been developed under Linux and runs under Windows; most of it should run under MacOS as well, but it is not as well supported and you will have to build the package from sources. To run hierarchical models using WinBUGS, you need to have WINE set up on Linux; I'm not sure about MacOS.) The setup file is about 17M, and R takes up about 40M of disk space. If you are running an antivirus package that is configured to check the signatures of executable files before they run, make sure you turn it off or register the new files installed by R before proceeding. You may also have some difficulty downloading packages if you have a firewall running on your computer — if you have trouble, you may want to (temporarily, at your own risk!) disable it.

Once you have downloaded and installed R, start the R program. The setup program should have asked whether you want to add a shortcut to the desktop or the Start menu: if you didn't, you will have to search for a file called Rgui.exe, which probably lives somewhere like Program Files
R
R-2.4.1
bin depending on what version of R you are using and where you decided to install it. R will open up a window for you with a command prompt (>), at which you can type R commands. (Don't panic.)

You can exit R by selecting File/Exit from the menus, or by typing q() at the command prompt. In general, if you want help on a particular command (e.g. uml) you can type a question mark followed by the command name (e.g. ?uml)

You will next need to install the mixstock package and two other auxiliary packages, over the WWW, from within R (you will need to maintain a connection to the internet for this piece, although it is also possible to do this step off-line). Within R, at the command prompt, type the following commands:

```
> install.packages("mixstock")
> install.packages("plotrix")
```

```
> install.packages("coda")
> install.packages("abind")
> install.packages("R2WinBUGS")
```

In each case, answer `y` to whether you want to delete the source files; you won't need them again. The first command specifies the location of the `mixstock` package (the other packages all come from the default source for R packages). The `install.packages` commands download and install packages.

(If you don't have a convenient internet connection, you can also download the .zip files corresponding to the different packages and install them by going to the Packages menu within R and choosing `Install from local zip file`.)

3 Loading the `mixstock` package and reading in data

Start every session with the `mixstock` package by typing

```
> library(mixstock)
```

at the command prompt; this loads the `mixstock` and auxiliary packages.

The package can read plain text data files that are separated by white space (spaces and/or tabs) or commas. If your data are in Microsoft Excel, you should export them as a comma-separated (CSV) file. If they are in Word, save them as plain text. The expected data format is that each row of data represents a haplotype, each column except the last represents samples from a particular rookery, and the last column is the samples from the mixed population. Each row and column should be named; your life will be simpler if the names do not have spaces or punctuation other than periods in them (a common convention in R is to replace spaces with periods, e.g. `North.FL` for "North FL"). Do not label the haplotype column; R detects the presence of column names by checking whether the first row has one fewer item than the rest of the rows in the file.

For example, a plain text file (with haplotype labels `H1` and `H2` and rookery labels `R1`–`R3`) could look like this:

```
  R1 R2 R3 mix
H1 1 2 3 4
H2 3 4 5 6
```

Or a comma-separated file could look like this:

```
R1,R2,R3,mix
H1,1,2,3,4
H2,3,4,5,6
```

If you have data from multiple mixed stocks, either put those data in a separate file or run them all together as columns of the same table (you will get a chance to specify how many sources and how many mixed populations there are):

```
R1,R2,R3,mix1,mix2
H1,1,2,3,4,7
H2,3,4,5,6,0
```

To read in your data, you first need to make sure that R knows how to find them. The best thing to do is to use the `File/Change working directory` option under the file menu to move to a directory you will use for analysis, which should contain the data files you want to use and will contain R's working files. Once you have changed to the appropriate directory, you can read in your data files and assign the data to a variable (for example) `mydata`:

```
> mydata <- read.table("lahanas98.dat")
```

if you are using space-separated data, or

```
> mydata <- read.csv("myturtles.csv")
```

if you have comma-separated values.

Here I'll use the `lahanas98raw` data that comes with the package:

```
> data(lahanas98raw)
> mydata <- lahanas98raw
```

To make sure that everything came out OK, type the name of the variable alone at the command prompt: e.g.

```
> mydata
```

to print out the data, or

```
> head(mydata)
```

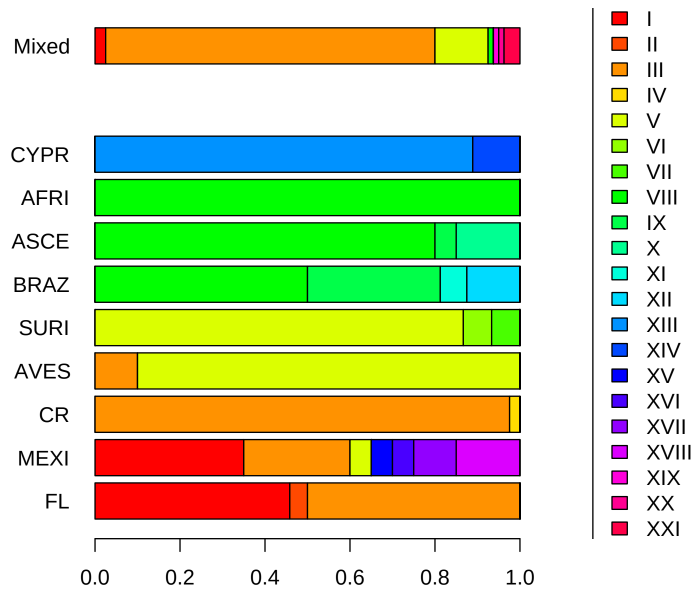


Figure 1: Basic plot of turtle mtDNA haplotype data.

```
     FL MEXI CR AVES SURI BRAZ ASCE AFRI CYPR feed
I    11    7  0    0    0    0    0    0    0    2
II    1    0  0    0    0    0    0    0    0    0
III  12    5 40    3    0    0    0    0    0   62
IV    0    0  1    0    0    0    0    0    0    0
V     0    1  0   27   13    0    0    0    0   10
VI    0    0  0    0    1    0    0    0    0    0
```

to print out just the first few lines, as shown above.

Next, convert your data to a form that the `mixstock` package can use with the `as.mixstock.data` command:

```
> mydata <- as.mixstock.data(mydata)
```

Once your data are converted to `mixstock.data` form, you can produce a summary plot of the data with `plot(mydata)` (Figure 1).

The default plot is a barplot, with the proportions of each haplotype sampled in each rookery represented by a separate bar; the mixed population data are shown as the rightmost bar.[1]

Before proceeding, you will need to "condense" your data set by (1) excluding any haplotype samples that are found only in the mixed population (which will break some estimation methods, and provide no useful information on turtle origins) and (2) lumping together all haplotypes that are found only in a single rookery and the mixed population (distinguishing among such haplotypes provides no extra information in our analyses, and may slow down estimation). You can do this by typing

```
> mydata <- markfreq.condense(mydata)
```

(To examine the condensed form of the data, you can print them by typing `mydata` at the command prompt, `head(mydata)` to see just the first few lines, or `plot(mydata)` to see the graphical summary [Figure 2].)

Some data are already entered in the package in the condensed format; you can access them using the `data()` command.

```
> data(lahanas98)
```

makes the haplotype frequency data from Lahanas et al. 1998 [5] available as variable `lahanas98`.

```
> data(bolten98)
```

gives you the loggerhead data from Bolten et al. 1998 [3] available as `bolten98`, already converted and condensed: `bolten98raw` gives you the raw table.

4 Stock analysis

Various methods of stock analysis are available.

4.1 Conditional and unconditional maximum likelihood

You can do standard conditional maximum likelihood (CML) analysis using `cml(mydata)`. If you want to save the results, you can save them as a variable that you can then print, plot, etc. (Figure 3)

[1]you can change from the default colors by specifying a `colors=` argument: e.g. if you have 10 haplotypes, `colors=topo.colors(10)` or `colors=gray((0:9)/9)`. See `?gray` or `?rainbow` for more information.

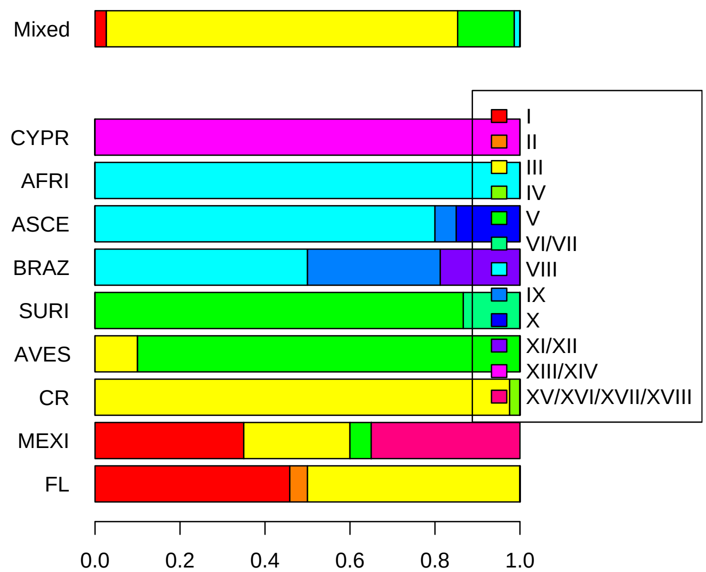


Figure 2: Condensed haplotype data from Lahanas 1998

```
> mydata.cml <- cml(mydata)
> mydata.cml

Estimated input contributions:
          FL         MEXI           CR         AVES         SURI         BRAZ
5.463021e-02 9.453698e-05 7.833919e-01 1.485493e-01 1.333410e-06 1.333277e-06
        ASCE         AFRI         CYPR
1.333144e-06 1.332877e-02 1.333010e-06

Estimated marker frequencies in sources:
(cml: no estimate)

method: cml

> plot(mydata.cml)
```

When you print CML results, R will tell you there is no estimate for the rookery frequencies, because CML assumes that the true rookery frequencies are equal to the sample rookery frequencies, rather than estimating the rookery frequencies independently.

The default plot for estimation results plots points specifying the estimated proportions of the mixed population contributed by each rookery (to plot this with a logarithmic scale for the vertical axis, use `plot(mydata.cml,log="y")`).

Standard unconditional maximum likelihood analysis (UML) takes a little longer, but is equally straightforward:

```
> mydata.uml <- uml(mydata)
```

UML estimates also include estimates of the true haplotype frequencies in each rookery, which are printed with the contribution estimates (print these results by typing `mydata.uml` on a line by itself). As with CML, you can plot the results with `plot(mydata.uml)`; by default this plot includes just the rookery contribution information. You can include the estimated haplotype frequencies in the rookeries in the graphical summary as follows:

```
> par(ask = TRUE)
> plot(mydata.uml, plot.freqs = TRUE)
> par(ask = FALSE)
```

(The `par` commands tell R to wait for user input, or not, between successive plots.)

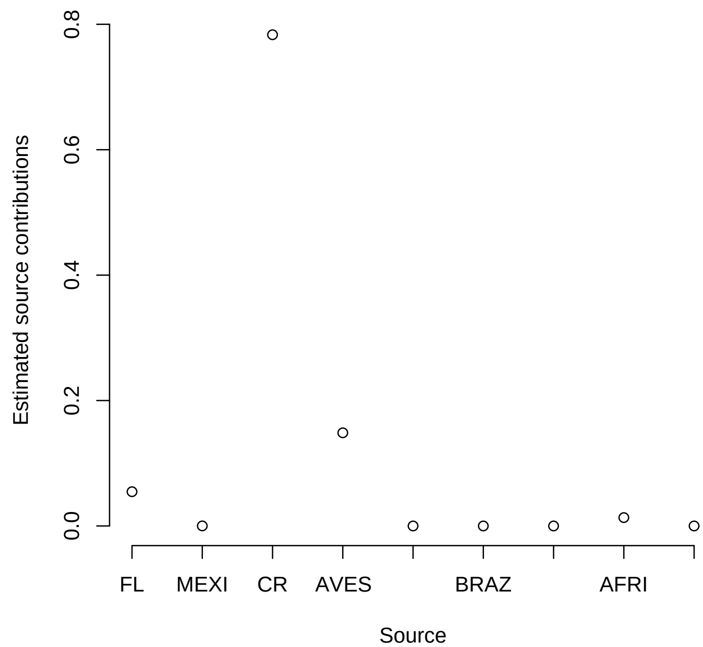


Figure 3: CML estimates for Lahanas 1998 data

4.2 Confidence intervals: CML and UML bootstrapping

```
> mydata.umlboot <- genboot(mydata, "uml")
```

will generate standard (nonparametric) bootstrap confidence intervals for a UML fit to `mydata`, by resampling the data with replacement 1000 times (by default). *This is fairly slow with a realistic size data set.* (You can ignore warnings about `singular matrix, returning equal contribs`, `Error in qr.solve`, etc..) You can find out the results by typing

```
> confint(mydata.umlboot)
```

```
                     2.5%        97.5%
contrib.FL   1.000000e-04 1.937642e-01
contrib.MEXI 8.172321e-05 9.999000e-05
contrib.CR   6.184032e-01 8.854842e-01
contrib.AVES 6.292138e-02 2.483440e-01
contrib.SURI 1.179836e-09 3.125456e-02
contrib.BRAZ 5.111485e-10 1.780757e-05
contrib.ASCE 1.598620e-13 2.008738e-05
contrib.AFRI 1.036273e-13 4.000358e-02
contrib.CYPR 1.779165e-13 2.142360e-05
```

4.3 Markov Chain Monte Carlo estimation

```
> mydata.mcmc <- tmcmc(mydata)

> mydata.mcmc
```

```
Estimated input contributions:
  contrib.FL contrib.MEXI   contrib.CR contrib.AVES contrib.SURI contrib.BRAZ
 0.055518267  0.009706668  0.777704826  0.105769897  0.036445990  0.003427765
contrib.ASCE contrib.AFRI contrib.CYPR
 0.004219192  0.005680010  0.001527386

Estimated marker frequencies in sources:
NULL

method: mcmc
prior strength: 0.1147742
```

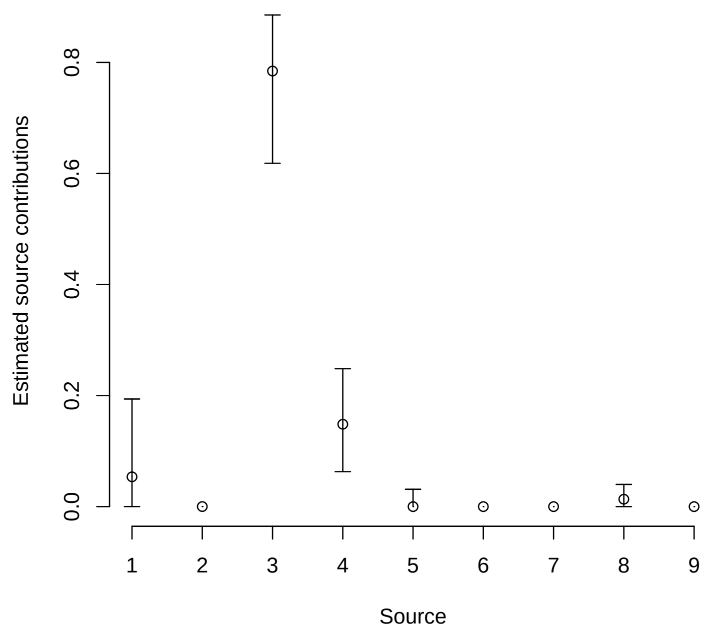


Figure 4: UML estimates with bootstrap confidence limits for Lahanas 1998 data

```
> confint(mydata.mcmc)

                     2.5%      97.5%
contrib.FL   2.009853e-11 0.23823757
contrib.MEXI 1.726347e-17 0.07512486
contrib.CR   5.956080e-01 0.89165907
contrib.AVES 3.616006e-10 0.22608667
contrib.SURI 7.363441e-16 0.17303709
contrib.BRAZ 1.664703e-16 0.02785796
contrib.ASCE 8.067783e-17 0.03001117
contrib.AFRI 3.820586e-15 0.03642586
contrib.CYPR 9.118769e-18 0.01506706

> plot(mydata.mcmc)
```

do the standard things: print the results, show confidence intervals, plot the results. (By default the information on haplotype frequencies in rookeries is not saved — it tends to be voluminous — and so this does not show up in the MCMC results.)

4.4 Convergence diagnostics for MCMC

When you are running MCMC analyses, you have to check that the Markov chains have *converged* (i.e. that you've run everything long enough for a reliable estimate).

4.4.1 Raftery and Lewis

The command

```
> diag1 = calc.RL.0(mydata)
```

runs *Raftery and Lewis* diagnostics on your data set: these criteria attempt to determine how long a single chain has to be in order for it to give "sufficiently good" estimates. This function actually runs an iterative procedure, repeating the chain until the R&L criterion is satisfied.

The results consist of two parts:

- `diag1$current` gives the diagnostics for the last chain evaluated. These diagnostics consist of the predicted required length of the "burn-in" period (a transient that is discarded); the total number of iterations

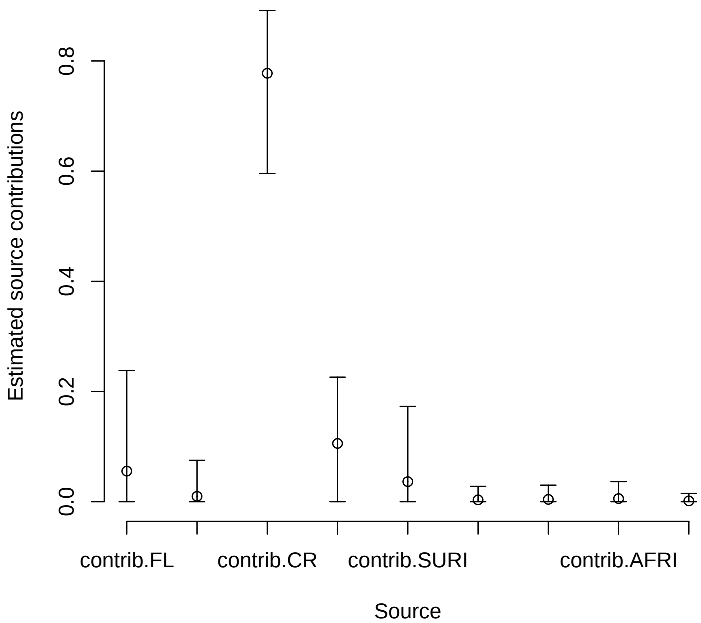


Figure 5: MCMC estimates with confidence limits for Lahanas 1998 data

required; a lower bound on the total number required; and a "dependence factor" that tells how much correlation there is between subsequent values in the chain (see `?raftery.diag` for more information). Here are the first few lines of `diag1$current`:

```
> head(diag1$current)
```

```
              Burn-in Total Lower bound Dependence factor
contrib.FL         18  1521         235              6.47
contrib.MEXI       14   926         235              3.94
contrib.CR         28  1804         235              7.68
contrib.AVES        4   312         235              1.33
contrib.SURI       15  1230         235              5.23
contrib.BRAZ        5   367         235              1.56
```

- `diag1$suggested` gives the history of how long each suggested chain was as we went along: the iterations stop once suggested >current, but note that there is a lot of variability in the results.

```
> diag1$history
```

```
iteration Current Suggested
        1     500       647
        2     647      3882
        3    3882      1804
```

4.4.2 Gelman and Rubin

The command

```
> diag2 = calc.GR(mydata)
```

tests the *Gelman-Rubin* criterion, which starts multiple chains from widely spaced starting points and tests to ensure that the chains "overlap" — i.e., that between-chain variance is small relative to within-chain variance. The general rule of thumb is that the criterion should be below 1.2 for all parameters in order for the chain to be judged to have converged properly. [4].

5 Hierarchical models

To install WinBUGS, go to http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml and follow the instructions there to download and install WinBUGS version 1.4 and get a license key. Then make sure that you've installed the R2WinBUGS package.

You can use the pm.wbugs() command (with the same syntax as tmcmc above) to run basic mixed stock analysis. Use mm.wbugs() to run many-to-many analyses.

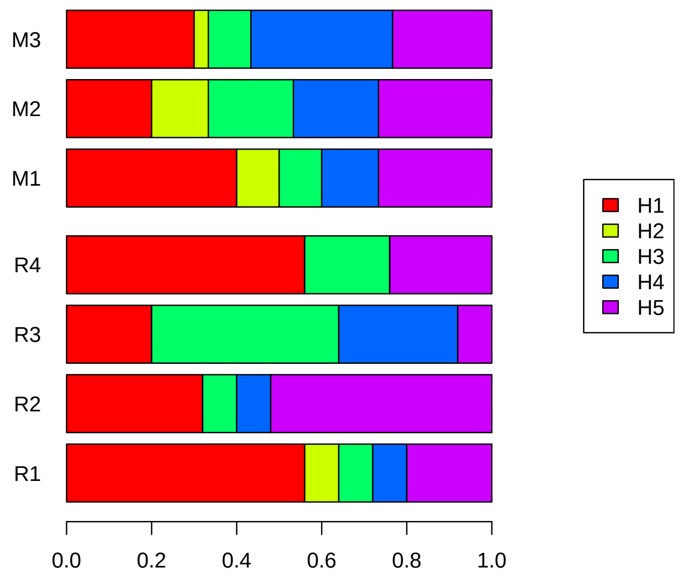
5.1 Many-to-many analysis

The simmixstock2 command does basic simulation of multiple-mixed-stock systems. At its simplest, it simply generates random uniform values for the haplotype frequencies in each rookery and the proportional contributions of each rookery to each mixed stock:

```
> Z <- simmixstock2(nsource = 4, nmark = 5, nmix = 3, sourcesize = c(4,
+     2, 1, 1), sourcesampsize = rep(25, 4), mixsampsize = rep(30,
+     3), rseed = 1001)
> Z

4 sources, 3 mixed stock(s), 5 distinct markers
Sample data:
   R1 R2 R3 R4 M1 M2 M3
H1 14  8  5 14 12  6  9
H2  2  0  0  0  3  4  1
H3  2  2 11  5  3  6  3
H4  2  2  7  0  4  6 10
H5  5 13  2  6  8  8  7

> plot(Z)
```



Now try to fit this via `mm.wbugs`:

```
> Zfit <- mm.wbugs(Z, sourcesize = c(4, 2, 1, 1))
```

Or, keeping the run in BUGS format for diagnostic purposes:

```
> Zfit0 <- mm.wbugs(Z, sourcesize = c(4, 2, 1, 1), returntype = "bugs")
```

This takes about 18.3 minutes to run with the default settings, which run 4 chains (equal to the number of sources) for 20,000 steps each. (There are two different versions of the BUGS code that can be used with `mm.wbugs`; in this particular case they give relatively similar answers and take about the same amount of time (`bugs.code="BB"` took 9.2 minutes), but if you're having trouble you might try switching from the default `bugs.code="TO"` to `bugs.code="BB"`.

Other important options when running `mm.wbugs` are:

- `n.iter`: the default is 20,000 iterations per chain, with the first half used as burn-in (`n.burnin=floor(n.iter/2)`); this may be conservative, and could take a long time with realistically large data sets. Use

CODA's diagnostics as described above (`raftery.diag`, `gelman.diag`, etc.) to figure out an appropriate number of iterations.

- `n.chains`: equal to the number of sources by default, which may again be overkill. ([2] used three chains for an 11-source problem.)

- `inittype`: `"dispersed"` starts the chains from a starting point where 95% of the contributions are assumed to come from a single source; `"random"` starts the chains from random starting points. If `which.init` is specified, these sources will be used as the dominant starting points: for example, `mm.wbugs(...,n.chains=3,inittype="dispersed",which.init=c(1,5,7))` will start 3 chains with dominant contributions from sources 1, 5, and 7. If `which.init` is unspecified and `n.chains` is less than the number of sources, dominant sources will be picked at random.

- `returntype`: specifies what format to use for the answer. The default is a `mixstock.est` object that can be plotted or summarized like the results from any other mixed-stock analysis. However, for diagnostic purposes, it may be worth running the code initially with `returntype="bugs"` and using `as.mcmc.bugs` and `as.mixstock.est.bugs` to convert the result to either CODA format or mixstock format. Plotting bugs format and CODA format gives different diagnostic plots; CODA format can also be used to run convergence diagnostics such as `raftery.diag` or `gelman.diag`.

Plots from many-to-many runs:
Plot BUGS format diagnostics (plot not shown):

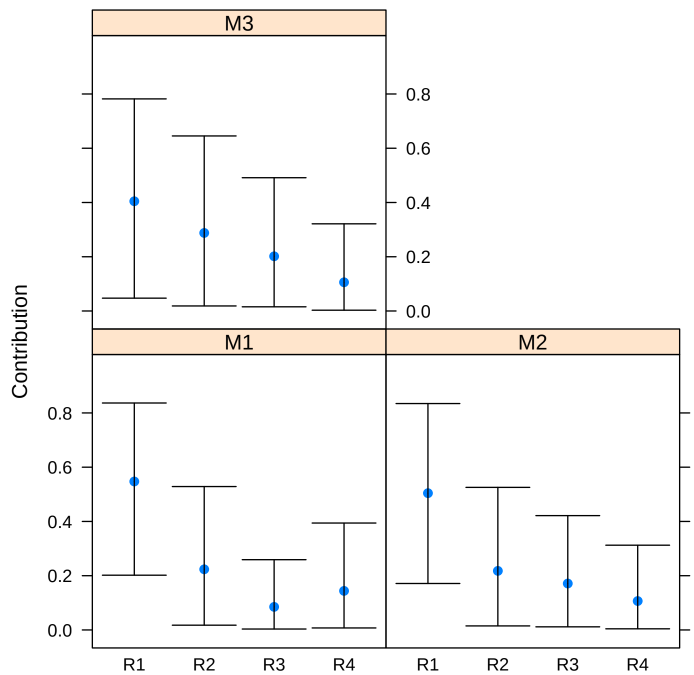
```
> plot(Zfit0)
```

Plot CODA diagnostics (plot not shown):

```
> plot(as.mcmc.bugs(Zfit0))
```

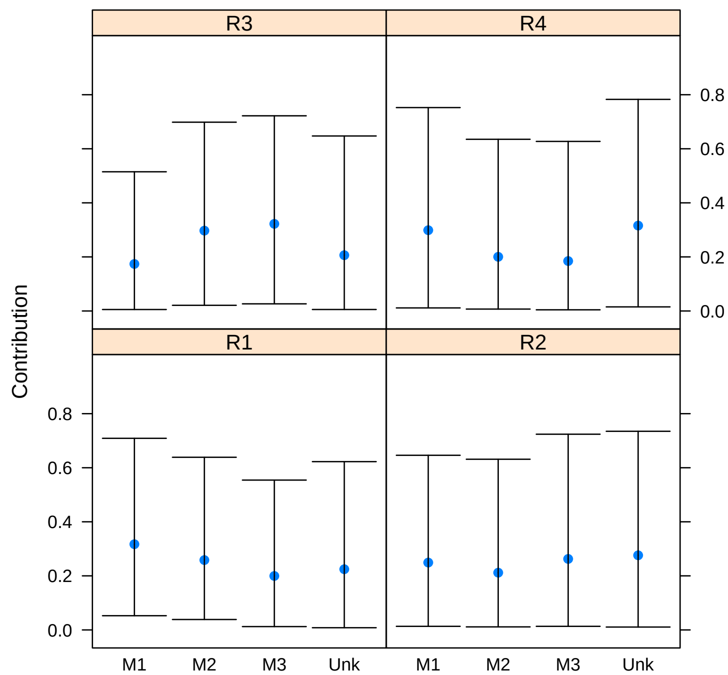
Plot results:

```
> print(plot(Zfit))
```



Source-centric form:

```
> print(plot(Zfit, sourcectr = TRUE))
```



Summary/confidence intervals:

```
> head(summary(Zfit))

4 sources, 3 mixed stock(s), 5 distinct markers
Sample data:
   R1 R2 R3 R4 M1 M2 M3
H1 14  8  5 14 12  6  9
H2  2  0  0  0  3  4  1
H3  2  2 11  5  3  6  3
H4  2  2  7  0  4  6 10
H5  5 13  2  6  8  8  7

Estimates:

Mixed-stock-centric:
                           2.5%     97.5%
M1.R1 0.5473780 0.201795000 0.8366150
M1.R2 0.2235784 0.017553250 0.5286050
```

```
M1.R3 0.0850429 0.003377650 0.2590050
M1.R4 0.1440014 0.007369775 0.3941075
M2.R1 0.5043251 0.171260000 0.8346125
M2.R2 0.2178163 0.014860500 0.5255300
M2.R3 0.1712309 0.011442625 0.4215025
M2.R4 0.1066277 0.004133800 0.3124100
M3.R1 0.4046099 0.047320750 0.7818925
M3.R2 0.2877887 0.018549000 0.6452925
M3.R3 0.2017308 0.015441500 0.4913425
M3.R4 0.1058681 0.002893225 0.3213625

Source-centric:
                        2.5%     97.5%
R1.M1  0.3171615 0.052617250 0.7088300
R1.M2  0.2584727 0.038580500 0.6387150
R1.M3  0.1997042 0.012389250 0.5542900
R1.Unk 0.2246619 0.008175600 0.6225700
R2.M1  0.2492528 0.013269500 0.6460600
R2.M2  0.2118914 0.011240250 0.6314400
R2.M3  0.2626997 0.013295500 0.7239800
R2.Unk 0.2761556 0.010689750 0.7348300
R3.M1  0.1740109 0.005432050 0.5149200
R3.M2  0.2972163 0.020928500 0.6983675
R3.M3  0.3223322 0.026362250 0.7219875
R3.Unk 0.2064394 0.005509450 0.6473575
R4.M1  0.2988757 0.011309500 0.7524525
R4.M2  0.2004035 0.007036625 0.6351050
R4.M3  0.1847740 0.004338375 0.6272475
R4.Unk 0.3159484 0.015142750 0.7827350
$data
4 sources, 3 mixed stock(s), 5 distinct markers
Sample data:
   R1 R2 R3 R4 M1 M2 M3
H1 14  8  5 14 12  6  9
H2  2  0  0  0  3  4  1
H3  2  2 11  5  3  6  3
H4  2  2  7  0  4  6 10
H5  5 13  2  6  8  8  7

$fit
```

```
$fit$input.freq
          R1        R2        R3        R4
M1 0.5473780 0.2235784 0.0850429 0.1440014
M2 0.5043251 0.2178163 0.1712309 0.1066277
M3 0.4046099 0.2877887 0.2017308 0.1058681

$fit$source.freq
NULL

$fit$sourcectr.freq
          M1        M2        M3   Unknown
R1 0.3171615 0.2584727 0.1997042 0.2246619
R2 0.2492528 0.2118914 0.2626997 0.2761556
R3 0.1740109 0.2972163 0.3223322 0.2064394
R4 0.2988757 0.2004035 0.1847740 0.3159484


$resample.sum
            mean   median         sd       Q02.5       Q05      Q95     Q97.5
M1.R1  0.5473780 0.553600 0.16110594 0.201795000 0.2595000 0.799230 0.8366150
M1.R2  0.2235784 0.204450 0.13855505 0.017553250 0.0260570 0.474390 0.5286050
M1.R3  0.0850429 0.068225 0.06931447 0.003377650 0.0065684 0.233520 0.2590050
M1.R4  0.1440014 0.126050 0.10132943 0.007369775 0.0140235 0.334100 0.3941075
M2.R1  0.5043251 0.503550 0.16885282 0.171260000 0.2143150 0.782120 0.8346125
M2.R2  0.2178163 0.204700 0.13563086 0.014860500 0.0260610 0.468530 0.5255300
M2.R3  0.1712309 0.154500 0.10862593 0.011442625 0.0224255 0.379490 0.4215025
M2.R4  0.1066277 0.087870 0.08396023 0.004133800 0.0089415 0.272715 0.3124100
M3.R1  0.4046099 0.399100 0.20215962 0.047320750 0.0800140 0.738310 0.7818925
M3.R2  0.2877887 0.274750 0.17065027 0.018549000 0.0354680 0.596360 0.6452925
M3.R3  0.2017308 0.184400 0.12848814 0.015441500 0.0253800 0.435915 0.4913425
M3.R4  0.1058681 0.084805 0.08726567 0.002893225 0.0070214 0.287610 0.3213625
R1.M1  0.3171615 0.292000 0.17826667 0.052617250 0.0752155 0.651575 0.7088300
R1.M2  0.2584727 0.225500 0.16266044 0.038580500 0.0508010 0.574510 0.6387150
R1.M3  0.1997042 0.161000 0.15118056 0.012389250 0.0201575 0.504265 0.5542900
R1.Unk 0.2246619 0.185450 0.17268818 0.008175600 0.0161995 0.551420 0.6225700
R2.M1  0.2492528 0.221400 0.17715397 0.013269500 0.0206450 0.579150 0.6460600
R2.M2  0.2118914 0.175000 0.16305664 0.011240250 0.0201865 0.522395 0.6314400
R2.M3  0.2626997 0.223000 0.19132121 0.013295500 0.0223965 0.634180 0.7239800
R2.Unk 0.2761556 0.241950 0.19892308 0.010689750 0.0219895 0.644830 0.7348300
R3.M1  0.1740109 0.135750 0.14152211 0.005432050 0.0128130 0.451170 0.5149200
```

```
R3.M2  0.2972163 0.272700 0.18146115 0.020928500 0.0434125 0.629540 0.6983675
R3.M3  0.3223322 0.298150 0.19033388 0.026362250 0.0460470 0.656430 0.7219875
R3.Unk 0.2064394 0.158350 0.17602759 0.005509450 0.0108000 0.571265 0.6473575
R4.M1  0.2988757 0.256650 0.20717218 0.011309500 0.0235090 0.687640 0.7524525
R4.M2  0.2004035 0.150150 0.16932025 0.007036625 0.0121855 0.531450 0.6351050
R4.M3  0.1847740 0.134400 0.16408396 0.004338375 0.0093100 0.520820 0.6272475
R4.Unk 0.3159484 0.269400 0.21798576 0.015142750 0.0292240 0.729235 0.7827350
```

(check this!)

6 Quick start

- Download and install R from CRAN (find the site closest to you at `http://cran.r-project.org/mirrors.html`; go to "Precompiled binary distributions" and from there to the base package; pick your operating system; download the setup program; and run the setup program).
- Start R.
- From within R, download and install the `mixstock` package and auxiliary packages:

```
> bbcontrib <- "http://www.zoo.ufl.edu/bolker/R/windows"
> install.packages("mixstock", contriburl = bbcontrib)
> install.packages("plotrix")
> install.packages("coda")
> install.packages("abind")
> install.packages("R2WinBUGS")
```

  (This installation procedure needs to be done only once, although the `library` command below, loading the package, needs to be done for every new R session.)

- Load the package: `library(mixstock)`
- Load data from a comma-separated value (CSV) file, convert to proper format, and condense haplotypes:

```
> mydata <- hapfreq.condense(as.mixstock.data(read.csv("myfile.dat")))
```

- analyze, e.g:

```
> mydata.mcmc <- tmcmc(mydata)
> mydata.mcmc
> intervals(mydata.mcmc)
> plot(mydata.mcmc)
```

7 To do

- read.csv/read.table + as.mixstock.data combined into a single read.mixstock.data command? (also incorporate hapfreq.condense as a default option)
- `print.mixstock.est` could print sample frequencies instead of saying "no estimate" for CML
- MCMC section could be cleaned up considerably, explained better, R&L parameters not hard-coded, more efficient — don't re-run chains every time
- incorporate rookery sizes in data
- keep CODA objects or potential for CODA plots in MCMC results
- make MCMC convergence process more efficient: more explanation
- add hierarchical models????
- describe fuzz and bounds parameters on CML/UML, E-M algorithm
- plot(...,legend=TRUE) doesn't work for CML. add unstacked/beside=TRUE option to plot.mixstock.est
- incorporate source size data as part of data object
- some functions don't work with uncondensed data: fix or issue warning
- use `HPDinterval` from CODA for confidence intervals, rather than quantiles?

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