SOFTWARE TOOL ARTICLE

Towards reproducible research: From data analysis (in R) to a typeset laboratory notebook (as .pdf) using the text editor Emacs with the 'mp' package [version 1; peer review: 2 not approved]

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Abstract

Much scientific research makes use of commonly available 'office' software. While numerous more fully-featured open-source alternatives exist, the integration of diverse tools and platforms which their use often entails can be challenging. The mp package for Emacs aims to bring together a number of these elements with the goal of simplifying the process of converting an .R file, as used for data analysis, to a nicely formatted .pdf which includes the complete description of an experiment. We discuss the rationale for development of the package and illustrate its applications and options with a series of experiments from our laboratory.

Keywords

latex , R , knitr , emacs , reproducible research , beta-hydroxybutyrate , melanoma , brain metastases

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Introduction

Motivation

One of the primary goals of any experimental research is to produce a nicely typeset document which explains the methods and results. This should be sufficient to allow the reader to recreate the work and thus to verify the results (given the correct tools).

In practice, much research is documented by adapting existing ‘office’-type software for this purpose (Microsoft, OpenOffice etc.). While there is much to be said for the ease of use of these techniques, they are not ideally suited to the purpose.

In particular, those that employ a ‘point-and-click’ graphical user interface (GUI) make it impossible to recreate these steps (mouse movements and clicks). The options for generating graphs and analyzing data are typically limited and often require the use of separate ‘third-party’ software for these steps (e.g. SPSS, GraphPad Prism). This again makes the reproduction of results a challenge.

There are many free and open source alternatives which are designed with the needs of the laboratory researcher in mind. Ease of use appears to be the principle reason for their lack of widespread use. The **mp** (for ‘*make-pdf*’) package came about as an attempt to bring elements from a number of these diverse sources together under one roof. It was also motivated by the repetitive nature of much laboratory research. Successive experiments often differ little in method, so that the analysis often uses the same techniques, with new experimental data being supplied each time.

Details of the experiment are documented and the R code for analysis is checked as usual; the **mp** package automates much of the rest of the process with one keystroke. Typesetting is performed using LaTeX, which has become the industry standard for publishers of scientific journals¹.

Emacs is the text editor which brings these methods together. Emacs itself has been criticized for lack of ease of use, although if used purely as a text/file editor, as in the examples here, it remains quite simple². Some familiarity with setting customizable variables is required when moving beyond the default settings for the **mp** package.

The examples below do assume some familiarity with R. The transition from familiar GUI-style data-analysis to terminal-based output may appear daunting at first. For those considering taking the plunge, we hope that these simple examples will help to illustrate how easy the R language can be to use. As a long-term investment, we feel that the time taken to become familiar with these methods is likely to be more than compensated by subsequent improvements in the speed and simplicity of workflow.

Existing solutions

There are already a number of tools useful in converting R source code to a nicely displayed .pdf file. The goal of the **mp** package is

to generate such .pdfs ‘at the touch of a button’. It allows for conversion of the source .R file to the final .pdf³. Further editing, when required, is performed on an intermediary (or ‘go-between’) file - either a no-web (.Rnw) or an .org file^{4,5}. **mp** can also generate documentation for an elisp package from the package’s own source code.

Currently, there are three widely used converters when generating a .pdf from an .R file. These are:

- **knitr** (‘knitter’).
- **Sweave** (‘S-weave’).
- Emacs’ own **Org** mode (herein org-mode).

Following the existing trend to name these tools after processes involved in fabric making, we refer to them collectively as ‘*entwiners*’.

An allied approach, converting from .org to .Rnw has also been implemented using the **ravel** package for Emacs⁶. Another similarly themed project is the **pander** package for R, which integrates R with the Haskell library **pandoc**^{7,8}. This could serve as an alternative to any of the above or be used in conjunction with them. For the sake of simplicity, the range of alternatives provided by the following three ‘entwiners’ appears sufficient for most needs.

Sweave. The oldest and best-supported of these converters is **Sweave**⁹. It is arguably the best integrated with R and remains the standard tool for R package developers writing accompanying vignettes. It remains widely used in generating statistical reports.

It does suffer from a number of limitations relative to its counterparts. In particular, the displayed code ‘as-is’ has little formatting or use of color.

Also, by default, only one figure per ‘chunk’ (i.e. block of code) is supported. Outputting multiple figures typically means writing to, and reading from files explicitly in R, which can be tedious to implement.

Knitr. The **knitr** package for R generates code for display which is arguably ‘prettier’ than **Sweave**¹⁰. It also allows for multiple figures per chunk. It also allows the chunks to be kept in a separate source file - as opposed to requiring them to be part of the .Rnw file. There are more options for the display of terminal output e.g. including error messages. Like **Sweave**, it can be used to build R package vignettes.

knitr already integrates well with some existing GUI-style .tex editors, particularly LyX. The latter is part of ‘Scientific Workplace’ (SW), which, like **mp**, tries to make life easier for the laboratory researcher by providing a simplified workspace. In the case of SW, a GUI is preferred to directly editing files¹¹.

org-mode. `.org` files are arguably easier/more intuitive to read and edit than `.tex`, particularly for users new to the latter. In particular, tables are much more straightforward to read, create and modify. Also, the use of ‘collapsible’ section headers makes it easy to see the structure of a document at-a-glance before focusing on one section for editing.

Org-mode in Emacs allows for the export of code chunks/blocks. Like **Sweave**, org-mode chunks suffer from the drawback that multiple figures per chunk are not supported by default.

While org-mode loses the attractive code printing of **knitr**, some worthy alternatives are provided by the LaTeX package **listings**¹². These include the option to include LaTeX maths markup in the code commentary, for example to display equations.

Org-mode allows for conversion/export to multiple file types. By default, **mp** converts `.tex` to `.pdf` but alternatives are straightforward, such as to `.html` or to `.MARKDOWN`.

In **mp**, the settings adopted for **listings** are modeled on those of the **knitr** defaults. We acknowledge that they are perhaps not as attractive. These are the same settings used for the code listings in this article.

An alternative to **listings** is the **minted** package for LaTeX. While probably more attractive for code display in general, its use involves a package for another language (**Pygments**, written for python). Also, outlining multi-page blocks with background coloring is tricky to automate.

On reproducible research

Much scientific research is reported in the form of a *summary* and it is not traditional for researchers to provide detailed accounts of the original experiments along with the original observations/results made at the time.

The classic quote on the subject is from Buckheit and Donoho¹³:

An article about computational science in a scientific publication is not the scholarship itself, it is merely advertising of the scholarship. The actual scholarship is the complete software development environment and complete set of instructions which generated the figures.

The same may well be said of biology. With a traditional print-form journal, the constraints of space limit the length of articles and thus providing content as a summary is sensible. However, the option to refer to online-only Supplementary material now obviates such impediments.

Such supplements may be valuable for those seeking to reproduce steps in the research. They may also be of interest to researchers working on closely related problems, particularly by saving time on preparatory work. Providing original data may allow such data to be combined with that from subsequent experiments. Also, analytic techniques beyond those used in the original paper may later be brought to bear by other researchers.

The details of individual experiments, particularly ‘pilot’ studies of an exploratory nature or those which yielded negative results are likely to be of limited interest to a general readership. Including these resources as supplements appears reasonable.

The supplements of this main paper illustrate the applicability of the **mp** software to a series of experiments from our laboratory which demonstrate the inhibitory effects of *beta*-hydroxybutyrate (BHB) on the growth and migration of a melanoma cell line, selected for its propensity to form brain metastases, *in vitro*.

Methods

Software implementation

Package details. **mp** is written in elisp, the native language of Emacs¹⁴. The package aims to automate the workflow associated with converting from `.R` files to `.pdfs`. Data analysis is done primarily from the `.R` file directly - in preference to the intermediary `.Rnw` or `.org` file, which can become cumbersome.

mp is ‘opinionated’ when generating intermediary files. Certain defaults have been chosen based on the authors’ experience, for example with the settings for the LaTeX **hyperref** package.

In order to streamline the whole process, most of the processing takes place *asynchronously*. That is, document editing can proceed as the Emacs terminal is not ‘frozen’ during the process of `.pdf` generation.

No support for caching is provided, although with short documents similar in scale to the examples below this should not result in much loss of performance. The time to compile is <10 secs with an Intel i5-2430M processor for all of the examples given.

The manual for the **mp** package is given as [Supplementary material](#) (this is generated from the code itself, using an `.org` intermediary file.).

The package defines the **mp** minor mode for Emacs. The only key-binding involves the function `mp-mp` with Ctrl-Alt-| (usually found above the ‘Return’ key; in Emacs parlance this is also known as C-M-|). This is intended as a gateway to all of the package functions, all of which can also be run individually (or ‘interactively’).

`mp-mp` will prompt for a filename; if none is supplied, it will look first at the current buffer. If this is not an `.R`, `.Rnw`, `.org.tex` or `.el` file it will select the appropriate file from the default-directory as that which has most recently modified. Thereafter it will search up the directory tree if no such file is found.

Instead of a filename, the single character 'p' may be given to display the appropriate `.pdf` associated with the current file or directory.

Control flow. A simplified flow diagram of the **mp** package is presented in Figure 1.

A more detailed diagram, which also shows the customizable variables, is available as [Supplementary material](#) (`mpFlow.pdf`). While the latter may appear complex, all of the functions and variables are accessed through the 'umbrella' `mp-mp` function so that in a typical use case there is no need to alter the default settings.

Figure 1. Flow diagram showing file types and converters in the **mp** package. The red arrow shows the default flow path.

For more experienced users, this use of multiple functions and variables allows for highly granular control, if desired.

Use case. A typical use-case for experimental work proceeds as follows:

1. Create a new directory to hold all the files below.
2. Draft the code for analysis in .R.
Arbitrary 'placeholder' data may be used at this stage.
3. Create a 'skeleton' .pdf from .R.
4. Use the intermediary file generated to draft the experimental protocol.
5. Complete the experiment.
6. Update the .R or intermediary file generated (.Rnw or .org) with the results of the experiment and the conclusions.
7. Generate the final .pdf from the intermediary file.

Typically, the user will begin with an .R file which contains a number of chunks of R code. These are designated by the header `## ---- chunkName`. This follows the convention introduced by **knitr** for naming chunks. Each chunk continues until the following chunk starts.

The chunks are processed by the option given by the customizable variable `mp-entwiner`, which is `knitr` by default.

If no .Rnw file exists in the current directory, one is created by `mp-skeleton-nw`. If an .Rnw file *does* exist, it is updated using

`mp-update-nw`; those chunks matching the names in the .R file are updated and new chunks are added if required.

The default workflow is shown in [Figure 1](#) by the path through the red arrows. Some of the pros and cons of different choices of `mp-entwiner` are shown in [Table 1](#).

'Hello world' with mp. We begin with the simplest use case. We create the directory `hello` and place the contents of [Listing 1](#) into `hello.R`. With this file open in Emacs, we then run `mp-mp` with the key combination `C-M-|`.

Listing 1: The file `hello.R`

```
## ---- Print hello
print("Hello world")
## ---- Here are two graphs
## here's a comment
plot(1:10, 10:100)
## this second graph should appear
## below the first
plot(1:10, sqrt(1:10))
```

The final product, `hello.pdf` is shown in [Figure 2](#).

The intermediary file `hello.Rnw` will be left open in Emacs for further modification and this is shown in [Listing 2](#). There is a lengthy preamble which sets defaults for multiple options, here in LaTeX and R (including **knitr**). Of course, most of this is not relevant to such a simple example.

Table 1. Choice of `mp-entwiner` when converting from .R to .pdf.

Method	Advantages	Disadvantages	Typical use case
knitr	Code display is 'easiest to read'	Default settings may conflict with other options in LaTeX preamble	More R code than documentation
Sweave	'Industry Standard' in R community Robust; tried and tested	More limited options than knitr More than one figure per chunk is challenging	'Traditional' format desired
Org	.org files easier to read/write than .tex, particularly simple tables Display multiple languages Math in code comments Export to HTML	Code display may not be as clear as others (particularly if used for output)	More documentation than code Source file (.org) needs to be easy to modify for collaborators User new to LaTeX

(2.1)

hello

Chris Dardis

Thursday 7th May, 2015

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0.2	Here are two graphs	1

0.1 Print hello

```
print("Hello world")  
  
## [1] "Hello world"
```

0.2 Here are two graphs

```
## here's a comment  
plot(1:10, (1:10)^2)  
## this second graph should appear  
## below the first  
plot(1:10, (1:10)^-1, type="b", col="blue")
```

(2.2)

(2.3)

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CONTENTS

3

Figure 2. The file hello.pdf.

Listing 2: The file hello.Rnw

```

%
\documentclass{article}
%
% modified from default setup for knitr
%
\usepackage[]{graphicx}
\usepackage[]{color}
\usepackage{framed}
% recommended with 'knitr'
\usepackage{alltt}
\usepackage{mathtools}
\usepackage[sc]{mathpazo}
\usepackage{geometry}
\geometry{verbose, tmargin=2.5cm, bmargin
    =2.5cm,
    lmargin=2.5cm, rmargin=2.5cm}
\setcounter{secnumdepth}{2}
\setcounter{tocdepth}{2}
\usepackage{url}
\usepackage{hyperref}
\hypersetup{unicode=true, pdfusetitle}
\hypersetup{bookmarks=true,
    bookmarksnumbered=true}
\hypersetup{bookmarksopen=true,
    bookmarksopenlevel=2}
\hypersetup{breaklinks=false, pdfborder={0 0
    1}}
\hypersetup{backref=false}
\hypersetup{colorlinks=true}
\definecolor{myDarkBlue}{rgb}{0, 0, 0.5}
\hypersetup{linkcolor=myDarkBlue}
\hypersetup{pdfstartview={XYZ null null 1}}
%
% Palatino family
\usepackage[T1]{fontenc}
\usepackage{mathpazo}
\linespread{1.05}
\usepackage[scaled]{helvet}
%
% other useful additions
%
%% for rerunfilecheck:
% no need to rerun to get outlines right
\usepackage{bookmark}
%% for nice tables
\usepackage{booktabs}
%% for e.g. \formatdate
\usepackage{datetime}
%% for SI units
\usepackage{siunitx}
%% for chemical symbols
\usepackage[version=3]{mhchem}
%% to use forced 'here'
%% e.g. \begin{figure}[H]
\usepackage{float}

%% for large numbers of floats
\usepackage{morefloats}
%% to keep floats in same section
\usepackage[section]{placeins}
%% for tables > 1 page
\usepackage{longtable}
%
%%-----
%
\begin{document}
%
% knitr chunks
<<setup, include=FALSE>>=
library(knitr)
### Set global chunk options
opts_chunk$set(
    eval=TRUE,
    ## text results
    echo=TRUE,
    results=c('markup', 'asis', 'hold', 'hide')
    [1],
    collapse=FALSE,
    warning=TRUE, message=TRUE, error=TRUE,
    split=FALSE, include=TRUE, strip.white=TRUE,
    ## code decoration
    tidy=FALSE, prompt=FALSE, comment='##',
    highlight=TRUE, size='normalsize',
    background=c('#F7F7F7', colors()[479], c
        (0.1, 0.2, 0.3))[1],
    ## cache
    cache=FALSE,
    ## plots
    fig.path=c('figure', 'figure/minimal-'),
    fig.keep=c('high', 'none', 'all', 'first',
        'last')[1],
    fig.align=c('center', 'left', 'right', 'default')[1],
    fig.show=c('hold', 'asis', 'animate', 'hide')[1],
    dev=c('pdf', 'png', 'tikz')[1],
    fig.width=7, fig.height=7, #inches
    fig.env=c('figure', 'marginfigure')[1],
    fig.pos=c('', 'h', 't', 'b', 'p', 'H')[1])
### Set R options
options(formatR.arrow=TRUE, width=60)
knit_hooks$set(inline = function(x) {
    ## if (is.numeric(x)) return(knitr::format
        _sci(x, 'latex'))
    highr::hi_latex(x)
})
## uncomment below to change theme
## knit_theme$get()
## opts_knit$set(out.format='latex')
## thm1 <- knit_theme$get('acid')
## knit_theme$set(thm1)
@
% knitr read chunks
<<readChunks, include=FALSE>>=
read_chunk('hello.R')

```



```
@
\title{hello}
\author{Chris Dardis}

\maketitle
% page numbers appear top-right
\pagestyle{headings}
\tableofcontents

\subsection{Print hello}

<<Print hello>>=
@

\subsection{Here are two graphs}

<<Here are two graphs>>=
@

%
%% \bibliographystyle{plain}
%% \bibliography{hello}

\end{document}
```

Examples of use. Some examples more relevant to the laboratory follow. These are summarized in [Table 2](#). These are all available as [Supplementary material](#) and we encourage the reader to refer to the relevant files while reading the present article. Further details of the experimental work are given at the end of this section.

Table 2. Steps taken in generating examples.

	File	Entwiner	Edit	TeX	Final
1	8k	knitr	.Rnw*	LaTeX	.pdf*
2	16k	knitr	.Rnw*	LaTeX	.pdf*
3	16k2	Sweave	.Rnw*	XeTeX	.pdf*
4	32k	Org	.org*	LaTeX	.pdf*
5	32k2	Org	.org*	LaTeX	.html*
6	sa	knitr	.Rnw*	XeTeX	.pdf*

* = file available as [Supplementary material](#)

The ‘file-stems’ (i.e. the filename without a suffix) refer to the starting quantity of cells per 12-well plate. ‘sa’ in the last example stands for ‘scratch assay’.

The variable `mp-preamble` is used to set some defaults useful for typesetting documentation for the laboratory. For example, the LaTeX package `siunitx` is used for the correct display of scientific units¹⁵.

Example 1: 8k.pdf

Experiment: Establish number of cells required to establish sustained growth. Start with 8000 cells per 12-well plate. Our first aim was to establish the optimal number of B16-F1-Luc2-BR2 cells with which to seed a growth curve. We wished to establish sustained growth over the period of the experiment, without reaching a ceiling (i.e. maximum density).

Typesetting: default settings. The file `8k.R` is given in [Listing 3](#):

Listing 3: The file 8k.R

```
## ---- data
(df1 <- data.frame(Day = rep(0:4, each=3),
  Well = rep(LETTERS[1:3], 5),
  Count = c(rep(8e3, 3),
    193e3, 148e3, 159e3,
    78e3, 63e3, 79e3,
    33e3, 56e3, 33e3,
    13e3, 15e3, 10e3)))

## ---- stdError
stdErr <- function(x) sqrt(var(x)) / sqrt(
  length(x))
library(plyr)
(df2 <- ddply(df1, .(Day),
  function(df)
    c(mean = mean(df$Count),
      SE = stdErr(df$Count),
      'mean+SE' = mean(df$Count) +
        stdErr(df$Count),
      'mean-SE' = mean(df$Count) -
        stdErr(df$Count))))

## ---- plot
library(plotrix)
# confidence interval plot
with(df2, plotCI(x=Day, y=mean, ui=mean+SE, li
  =mean-SE,
  xlab="Day",
  ylab="Cell counts (Cells/ ml)",
  axes=FALSE))
axis(side=1, at=c(0:4))
axis(side=2, at=seq(0, 150e3, by=50e3))
box()
with(df2, lines(Day, mean))
```

With the above file open, we call `mp-mp` to generate the `a.pdf`; page 1 of the result is shown in part in [Figure 3](#).

As an intermediary step, the file `8k.Rnw` is generated. This is an R no-web file, which is essentially a typical `.tex` file with the additional code chunks inserted⁴. The term ‘web’ is not in reference to the world-wide web but to distinguish it from a contemporaneous approach to literate programming known as ‘web’.

8k0

c

Tuesday 4th August, 2015

Contents

0.1	data	1
0.2	stdError	1
0.3	plot	2

0.1 data

```
(df1 <- data.frame(Day = rep(0:4, each=3),
Well = rep(LETTERS[1:3], 5),
Count = c (rep (8e3, 3),
193e3, 148e3, 159e3,
78e3, 63e3, 79e3,
33e3, 56e3, 33e3,
13e3, 15e3, 10e3)))

##      Day Well  Count
## 1     0    A   8000
## 2     0    B   8000
## 3     0    C   8000
## 4     1    A 193000
## 5     1    B 148000
## 6     1    C 159000
## 7     2    A   78000
## 8     2    B   63000
## 9     2    C   79000
## 10    3    A   33000
## 11    3    B   56000
## 12    3    C   33000
## 13    4    A   13000
## 14    4    B   15000
## 15    4    C   10000
```

0.2 stdError

```
stdErr <- function(x) sqrt(var(x)) / sqrt(
length(x))
library(plyr)
```

Figure 3. Page 1 of the initial 8k.pdf.

We modify this to add details of the experimental protocol, written in LaTeX. The principal modifications are the use of 12pt for typesetting, which we adopt from hereon and the inclusion of some graphics with the LaTeX **subfig** package¹⁶.

With 8k.Rnw still open, we call mp-mp again to generate the final .pdf.

Example 2: 16k.pdf

Experiment: Number of cells required to establish sustained growth. 16000 cells per 12-well plate. Again, we tried to establish the optimal number of B16-F1-Luc2-BR2 cells with which to seed a growth curve. We increased the starting number of cells per well from 8000 to 16000. This appeared to be more promising.

Typesetting: using R to generate .tex output. We again begin with an .R file. This time however, we use R to create LaTeX output, rather than displaying the results of a chunk as R code. The output from R is interpreted as LaTeX directly - rather than as typical terminal output. We use the R **xtable** package for this purpose; there are many good alternatives¹⁷.

An example of R code generating output which can be interpreted by LaTeX is shown in Listing 4.

Listing 4: From the file 16k.R

```
## ---- Cell counts on Day 0
df1 <- data.frame(Cells_per_ml = c(392e3, 332e3,
  300e3))
library(xtable)
print(xtable(df1,
  caption="Cell counts on day 0",
  align=c("l", "c"),
  display=c("d", "e"),
  label="tab:ccd0"),
  booktabs=TRUE)
```

Again, the .Rnw file needs some modification. The main change is to set the knitr option results='markup' to

results='asis' to allow the results to be interpreted as .tex.

We can also restructure the document to allow the code output (here, in the form of tables or single lines of terminal output) to be mixed with the main text. This ensures there is no needless duplication of data entry, as is the case in the earlier example.

The output is shown as **Supplementary material** in 16k.pdf. Most of the materials and methods are unchanged from 8k.pdf.

Example 3: 16k2.pdf

Experiment: Demonstrate impaired growth in the presence of BHB. 16000 cells per 12-well plate. We sought to determine whether the growth of B16-F1-Luc2-BR2 cells would be impaired in the presence of BHB at a concentration of 10 mmol/L.

Due to the large standard error on day 4, no difference could be demonstrated via a t-test. However comparing linear models with and without treatment *did* show a significant effect (analysis of variance (ANOVA) $p = 0.048$).

Typesetting: Using Sweave and XeTeX. Here, we change the variable mp-entwiner to Sweave. We also change mp-latex to XeLaTeX to allow the use of an OpenType font (by default this is 'Linx Libertine'). While certain OpenType fonts are available as packages for LaTeX, XeTeX allows for a greater range and greater flexibility.

As in example 2, the output from each chunk is again typically interpreted as .tex (rather than displayed as R code) by including results=tex in mp-Sweave-opts.

Some modifications to the .Rnw file generated follow. There is one chunk where we prefer the output to retain typical R code formatting. Also we need to change the variable mp-Sweave-opts to fig=TRUE for the chunk which produces a plot.

This example also shows the value of LaTeX for typesetting equations - calculation of correct masses are nicely displayed and easy to follow. This is shown in Figure 4.

$$\begin{aligned} 1 \text{ mol} &= 126 \text{ g} & (1) \\ 10 \text{ mmol} &= 1.26 \text{ g} & (2) \\ 10 \text{ mmol/L} &= 1.26 \text{ g/L} & (3) \\ &= 0.00126 \text{ g/mL} & (4) \end{aligned}$$

Thus 0.00126 g in 10 μ L added to 1 mL gives:

$$0.00126 \text{ g per } 1 \text{ mL} = 10 \text{ mmol/L} \quad (5)$$

Figure 4. Typeset equations, from 16k2.pdf.

Example 4: 32k.pdf

Experiment: Demonstrate impaired growth in the presence of BHB. 32000 cells per 12-well plate. As the preceding experiment was inconclusive, we tried the same technique again, this time using a higher number of cells to start with.

Unfortunately, no observations were taken on day 3 of the experiment. This meant that both the t-test on day 4 and the ANOVA comparing linear models were not significant, although the latter did come close ($p = 0.07$).

Typesetting: using Org. Changing mp-entwiner to Org allows us to use an .org file as the intermediary step.

The output from this process is given as 32k.pdf. Again, some minor modifications to the intermediary file are required. In this case we need to change the headers for the chunk which produces a plot to specify the output of graphics (as opposed to text) and to specify an external file for the graphical output.

We also change some of the results to latex and pp (pretty print) to give examples of different styles of output. 32k.pdf also features a printout of help for an R function.

Example 5: 32k2.pdf

Experiment: Demonstrate impaired growth in the presence of BHB. 32000 cells per 12-well plate. We repeated the preceding experiment and this time there was a conclusive difference between the cell counts in those grown with BHB vs. controls.

```
Null hypothesis: mock less than BHB.
Density (percentage of starting value) by treatment."),
  label="tab:tt")
```

	time	p_value
1	0	0.07
2	3	0.04
3	6	0.03
4	20	0.03

Table 3: Welch Two Sample t-test. 1-sided. Null hypothesis: mock less than BHB. Density (percentage of starting value) by treatment.

5.4 Standard error

For plotting, we first calculate the standard error at each time point:

$$SE_x = \frac{s}{\sqrt{n}} \quad (3)$$

where s is the sample standard deviation ($s = \sqrt{\text{variance}}$).

```
stdErr <- function(x) sqrt(var(x, na.rm=TRUE)) / sqrt(length(x))
```

Typesetting: Using Org to generate HTML output. This example shows how the .org intermediary can be converted to .html as an alternative to .pdf. Converting to HTML means we lose all but the simplest LaTeX commands and so the document needs to be written in a simpler style. While we lose some of the nice typesetting features of LaTeX, this simplicity has its own attraction.

These files are faster to produce and smaller than their .pdf counterparts (1/5 the size in this example). HTML is also likely to be simpler to integrate into an existing website and offers the possibility of almost 'real-time' reporting of experimental results.

Example 6: sa.pdf

Experiment: Demonstrate impaired cell mobility in the presence of BHB. Here we use a scratch assay¹⁸. A line is made in the center of the wells on a 12-well plate with confluent cell growth. Images taken at various timepoints are analyzed with freely available imageJ software. The distance between the cells on either side of the scratch decreases as they migrate back to the center. This corresponds to a decrease in 'image density' as measured by imageJ. This may be downloaded from:

<http://rsbweb.nih.gov/ij/download.html>

Typesetting: Emulating a laboratory notebook with XeTeX. The final example shows how a cursive font may be used to mimic a handwritten lab notebook. We set mp-entwiner to knitr and set mp-latex to XeTeX to allow the use of an unusual font. A sample is shown in Figure 5.

Figure 5. Cursive script in sa.pdf.

When combining multiple images on a single page, fitting them into the cells of a table can be helpful for clarity and this example features a number of such tables.

Operation

System requirements: this should work with any recent version of Emacs, which is platform independent (i.e. works on Windows, Linux, Mac-OS). Version ≥ 24.4 is recommended to allow for automated export of `.org` to `.tex`.

To export to HTML, the elisp package **htmlize** is required¹⁹. Links to the package source may be found at:

<http://emacs-wiki.org/emacs/Htmlize>

A recent installation of R (>3.0) and TeX (2013 and on) is also assumed. We used TeX Live 2013 for these examples.

The package should work with LuaTeX as well as LaTeX, although this has not been thoroughly tested.

Experimental work

The purpose of these experiments was to demonstrate an inhibitory effect of BHB on the growth and migration of a melanoma cell line *in vitro*. Details of the aims, methods, results and conclusions are given for each experiment separately (see Table 2).

A summary of these experiments would normally make up just one part of an article; further details of the individual experiments could be combined and made available as a supplement. We provide the experiments separately to provide a typical use-case for the **mp** package.

Cells. B-16 melanoma cells were obtained from American Type Culture Collection (ATCC). To facilitate quantitative measurement of tumor growth, they were modified as described previously²⁰. Briefly, the cells were stably transfected with the gene encoding luc2 (luciferase) using the pGL4.51 [luc2/CMV/Neo] vector (Promega Corp, Madison, WI) and FuGENE 6 Transfection Reagent (Roche Applied Science, Indianapolis, IN) following conditions specified by the manufacturer. They were then injected into the heart (the right ventricle) of a mouse. These animals were sacrificed when bioluminescence was detected in the brain. Cells metastatic to the brain were recovered and were put into culture.

The same procedure was repeated again (injection into mouse heart and recovery of brain metastases). These cells are designated B16-F1-Luc2-BR2.

Animal handling. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of St. Josephs Hospital and Medical Center (protocol number 334

(A3510-01)). All surgery was performed under ketamine/xylazine anesthesia, and every effort was made to minimize suffering.

Effects of BHB on growth. Firstly, it was necessary to determine the optimal starting number of cells to use for a growth curve. If the cells are plated too sparsely, they have a tendency not to grow. Likewise if the concentration is too great to start with, then this will make any difference due to the effect of BHB difficult to see. This is complicated further by growing the cells in a circular well - the cells are in closer communication close to the center and more separated close to the edge. In choosing a starting number, we were guided primarily by prior work in our own laboratory.

To determine whether BHB would inhibit cell growth, we chose a concentration of 10 mmol/L, again based primarily on our prior experience. Under normal conditions, serum BHB has an upper reference limit of 0.3–0.5 mmol/L. Tests of BHB concentration (for patients) are available commercially and can be used in by those adhering to the ketogenic diet. This diet is most commonly employed in the treatment of drug-resistant epilepsy, where serum BHB concentration has been shown to correlate with the degree of seizure control²¹. In this study of 74 children, median levels were 8–10 mmol/L.

We chose a concentration of 10 mmol/L in order to be certain that an effect was present, before considering whether lower levels would be similarly efficacious.

Effects of BHB on migration. The scratch assay is a simple and widely used test to assess cell migration. Details of a typical protocol for the technique are available¹⁸. Using the same cell line, we sought to illustrate impaired migration in the presence of BHB.

Results

As shown in Figure 6, we were able to demonstrate a difference in growth rates with an initial concentration of cells of 32,000 per well and a BHB concentration of 10mmol/L. This is shown in full in 32k2.html.

We were also able to demonstrate a decrease in cell migration using the scratch assay, as shown in Figure 7, which is taken from sa.pdf.

Discussion

The inhibitory effects of BHB on the growth of neoplastic cells has now been observed in a number of cells lines in our laboratory and those of other investigators²². Similarly it has been shown to impair apoptosis and necrosis of the L929 mouse cell line (taken from normal subcutaneous areolar and adipose tissue)²³.

Impairment of growth of neoplastic cells due to ketones has been suggested to be in part due to the polyacetylation of histones²⁴. Ketone bodies also impair glycolysis, on which neoplastic cells are

Figure 6. Effect of BHB (10 mmol/L) on the growth of B16-F1-Luc2-BR2 cells. Taken from 32k2 .html (Supplementary material).

more heavily dependent. This occurs in part through inhibition of the activity of phosphofructokinase and hexokinase^{25,26}.

The finding that inhibition of growth occurs in a cell line selected for their propensity to metastasize to the brain is novel. Brain metastases are a significant contributor to morbidity and mortality in patients with cancer²⁷. We feel that this approach deserves further investigation. In particular, it would be of interest to determine whether the same phenomenon would be observed in breast and lung cancer cell lines with a propensity to metastasize to the brain. It would also be interesting to determine whether the inhibitory effects of BHB on cancer cells are evident *in vivo* when ketones

are given as a dietary supplement with a relatively normal diet - as opposed to the ketogenic diet, which may be challenging to adopt for patients affected by cancer.

Conclusions

We have demonstrated here that BHB, at a concentration of 10 mmol/L, is inhibitory to the growth and migration of B16-F1-Luc2-BR2 melanoma cells that were selected for their propensity to form brain metastases.

The **mp** package appears to be a useful addition to the tools available for facilitating reproducible research. Future development will

Figure 7. Effect of BHB (10 mmol/L) on the migration of B16-F1-Luc2-BR2 cells. Taken from sa.pdf (Supplementary material).

likely include increased support for the LaTeX package **biblatex**. Collaboration and suggestions for improvement are welcome. Please try to address these to the development site on GitHub if possible.

Data availability

The datasets for all experiments are available as part of the Supplementary material.

One of the primary motivations for developing this package was to facilitate sharing of datasets such as these.

Software availability

Source code

<https://www.github.com/dardisco/mp>

Archived source code as at the time of publication

<http://dx.doi.org/10.5281/zenodo.2118345>

Software license

GPL ≥ 3

Author contributions

CD - carried out the experiments (except for the scratch assay), wrote the software, performed the data analysis and drafted the manuscript.

ECW - carried out the scratch assay experiment; he was also closely involved in the design and execution of the other protocols.

ACS - supervised the experimental protocols and interpreted the results.

All authors have revised and approved the final manuscript.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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www.ssbtr.org

Supplementary material

Manual for mp package. Documentation, including all functions and variables in the package. Generated from the source code. This package is 'self-documenting'. `mp.pdf` is the manual for the package, which was generated with `mp-el-tex`. The option `INCLUDESOURCE` is set to `t`, so that the source code is shown for all functions. The intermediary file is `.org` which allows package documentation to be nicely displayed in list or table format using TeX, as appropriate.

[Click here to access the data.](#)

Flow diagram for mp package. An image, showing the control flow for the package, including file types, functions and customizable variables.

[Click here to access the data.](#)

Establish a 5-day growth curve starting with 8,000 cells/well. Example 1; default settings. Intermediary `.Rnw` file, typeset with knitr.

[Click here to access the data.](#)

<http://dx.doi.org/10.5256/f1000research.6800.s97727>

Establish a 5-day growth curve starting with 16,000 cells/well. Example 2; using R to generate LaTeX output. Intermediary `.Rnw` file, typeset with knitr.

[Click here to access the data.](#)

<http://dx.doi.org/10.5256/f1000research.6800.s97728>

Effects of BHB on growth starting with 16,000 cells/well. Example 3; using Sweave. Intermediary `.Rnw` file, typeset with Sweave (and XeTeX).

[Click here to access the data.](#)

<http://dx.doi.org/10.5256/f1000research.6800.s97729>

Effects of BHB on growth starting with 32,000 cells/well. Example 4; using org-mode. Intermediary `.org` file, typeset with org-mode and listings.

[Click here to access the data.](#)

<http://dx.doi.org/10.5256/f1000research.6800.s97730>

Effects of BHB on growth starting with 32,000 cells/well. Example 5; using org-mode to generate .html output. Intermediary .org file, typeset with org-mode and listings.

[Click here to access the data.](#)

<http://dx.doi.org/10.5256/f1000research.6800.s97731>

Effects of BHB on migration, via scratch assay. Example 6; using a cursive font and placing images in a table. Intermediary .Rnw file, typeset with knitr (and XeTeX).

[Click here to access the data.](#)

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Frank Harrell

Department of Biostatistics, Vanderbilt University, Nashville, USA

I cannot approve this article in its current form. The authors may be able to undertake a major revision that makes the paper acceptable.

I realize that this is a subjective opinion, but I just cannot agree with the software implementation chosen by the authors, for the following reasons:

1. Even though I've been using Emacs for more than 20 years and am an advocate of it for the user productivity it brings, I cannot agree that Emacs is the best choice of platform for this application. I say that primarily because RStudio provides the most elegant integrated development environment for R and it is elegantly tied to LaTeX and Markdown, two engines that the authors rightfully advocate.
2. The idea of hiding various complexities from researchers is a noble one, but this can be accomplished in more flexible ways using LaTeX packages. For example, a new Github project at <https://github.com/harrelfe/rlatex> developed by the reviewer provides LaTeX packages spaper and knitr that streamline reproducible LaTeX reports. Another way to accomplish this is to write R functions that output the needed LaTeX preamble.

The authors refer to both Sweave and knitr but knitr has now completely replaced Sweave. In addition, knitr provides an elegant way to specify figure size and captions.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 08 Mar 2016

Chris Dardis, Barrow Neurological Institute, Phoenix, USA

Apologies for the delay in responding. Thank you for this review; we appreciate the points you raise and feel the article has been improved in addressing these.

1) I (first author) began with RStudio and used the platform for at least 2 years. Ultimately, I moved entirely to Emacs as I found it to be more productive. While not a fully fledged 'integrated development environment', I found this to be more than compensated for by speed and simplicity, particularly in regards to text editing. Most of my work takes place within R; I did feel that integration with LaTeX within Emacs left something to be desired; hence the current package, which allows me to compile documents without leaving Emacs or 'freezing' my R session/ text editing while waiting for a document to compile.

I do acknowledge that RStudio may be a more suitable choice for a particular user. The article has been modified to reflect this; for those who are new to EMACS *and* R/LaTeX integration, I agree that RStudio is likely to be the best initial choice.

We are not recommending 'mp' as the 'best' way to achieve this integration but rather as a viable alternative to RStudio. Surely having a greater range of tools available can do no harm?

2) I did consider developing a package in LaTeX or R for this end. I was not aware of the tools you yourself have created and am grateful to you for bringing them to my attention; I have referenced these in the main paper. I discuss their merits in the new 'Allied approaches' section in the paper. As far as elisp goes, I think it is well suited for the task at hand; not to say that everything could not be done with R or LaTeX. In the case of the former, its facilities for processing text/ strings are not, in my view, as simple or as developed as those available in elisp. In the case of the latter, I find development in a non-interpreted language to be slower and more challenging.

"knitr has now completely replaced Sweave" - I disagree; while this may be the case for most practical purposes, Sweave retains the support of R-core and I am not aware of plans for knitr to receive such support. Given the amount of 'legacy' documents generated using Sweave, it is hard to see this changing; thus it will continue to remain important, if increasingly for historical reasons. I agree that knitr should be recommended for most tasks. Sweave does have the advantage of producing .tex files which are simpler to read; it is also a little faster.

"knitr provides an elegant way to specify figure size and captions" - agreed; which I have highlighted this in the main manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Report 10 September 2015

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Eric Schulte

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The report is insufficiently well written for me to evaluate the importance of the suggested tool.

The current version of this article is unclear as to (1) the functionality provided by mp, (2) the intended usage model of mp. It also (3) conflates an overly-detailed example with the main paper.

1. Is mp merely a translator from a .R format to multiple text document formats? Does mp perform any processing of the input data? Does mp provide stylistic control of the output from within a particular .R file or only through configuration variables?
2. I don't fully understand the usage model. Is prose written **within** the .R file itself, or in the automatically generated .org, .Rnw or .tex files? If the former what markup syntax does mp provide? If the later does subsequent evaluations of mp overwrite the manually edited intermediate file? These questions should be addressed early on in the article.
3. Finally, an inappropriate emphasis is placed on the "example" experiment. All portions of the example experiment should be typeset to indicate that they are example and their technical content is **not** part of this publication and has not been peer reviewed. As it reads the "results" and "discussion" sections seemed to discuss the content of the example more than the content of the tool.

While it seems very likely that the mp tool is a significant contribution to the practice of reproducible research, I am unable to evaluate its significance from this article.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 08 Mar 2016

Chris Dardis, Barrow Neurological Institute, Phoenix, USA

Apologies for the delay in responding. Thank you for this review; your points are well-taken. I have tried to clarify things by re-structuring the document and adding some new material. We hope that things will appear simpler if you get a chance to try out the package.

1 and 2) Functionality - we have tried to clarify this in the introduction and added a new 'What does mp do?' which should address these points.

3) The experiments *are* part of this publication; they are not present for purely illustrative purposes. The original focus of the manuscript was on the experimental work rather than the software; following discussion with the editorial office at F1000 research, we changed the focus to bring the software to the foreground (which is likely to be of more general interest).

Arguably, the experiments should have been the subject of a separate paper. However, the experiments are short and simple and some 'negative' results are included. Thus we felt it would be more appropriate to include both together (avoiding needless duplication).

Reporting novel methods with new experimental work is reasonably common; it is hard to see how this alone would lead to the conclusion that the material is unworthy of publication.

Understandably, it has been challenging to find reviewers willing to assess the quality of the software *and* of the experiments. It would be reasonable to consider the experimental work 'not peer reviewed' at present if you feel that this lies outside your expertise.

Competing Interests: No competing interests were disclosed.

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