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## **C3.2.0 BioInformatics Toolbox**

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local pool because of evolved differences in phenotype and are dominated by competitive exclusion rules, or evolved similarities in phenotype and dominated by habitat filtering rules (Webb 2000). Outside the community paradigm, there is also the simple question of whether an area has  $x$  species because they moved there or evolved there (Ricklefs and Schluter 1993). These questions can all be addressed by the specialist and non-specialist alike with a tool that can integrate phylogenies and spatial data, linked via a user-controlled list of taxon identities.

Macroecology is a loosely defined but vibrant new field at the interface between ecology, biogeography and macroevolution (Brown 1995). As most frequently practiced, macroecology involves documenting and analysing the distribution of taxon richness patterns (Figure 3) across large areas of space (Gaston 2000; Cardillo *et al.* 2005; Grenyer *et al.* 2006). Consequently, the main analytical tools are correlation statistics that can control for spatial non-independence (Diniz Filho and Bini 2005), and general linear modelling techniques which can deal with multivariate correlation structures with unusual distributions of error variance (Storch *et al.* 2006).

**Figure 3: Figure from Grenyer *et al.* (2006) showing the distribution of richness amongst vertebrate species across the world's surface. As designed here, the toolbox will be able to populate such grids by remotely querying distributional data sources such as GBIF.**

From the perspective of the toolbox user, macroecological analysis is a logical progression: once taxonomic and phylogenetic information are assembled in the toolbox, the final ingredient for conservation area selection (see below) is spatial information concerning the distribution of the taxonomic entities being considered. Consequently, if conservation area selection possible, then so is

macroevolutionary analysis for very little extra coding effort. Primarily, the functions required of the toolbox are to be able to take location information (either in the form of point localities, vector range maps or raster presence-absence data at arbitrary pixel location and dimension) and convert it into a richness surface at a resolution and location specified by the user. Handler functions to convert 2D richness surfaces to arbitrary 1D richness gradients would also be beneficial as such transformations are non-trivial at present. Note that it will also be necessary to warn the user when potential data error can be introduced in some of these processes, for example in the transposition of raster (image) data from one set of cell locations to another.

One further subgroup of analyses that is often considered a subset of the macroecological paradigm are the various phylogenetic comparative methods (PCMs). At the risk of generalising, PCMs are statistical techniques which convert the pattern of dissimilarities amongst taxa derived from a phylogeny into a prior expectation of dissimilarity amongst observed data for the species (Pagel 1999). This allows the partitioning of observed relationships amongst species traits into components that could be considered “phylogenetic” and “adaptive”; although more a more accurate depiction would be “well explained” and “not well explained” by a posited model of trait evolution (Freckleton and Harvey 2006). In essence, once the user has selected a model of evolution that they can justify for their particular research question, PCMs allow the users to see patterns and infer causation amongst their observations free from the confounding effects of inherited similarity of phenotype.

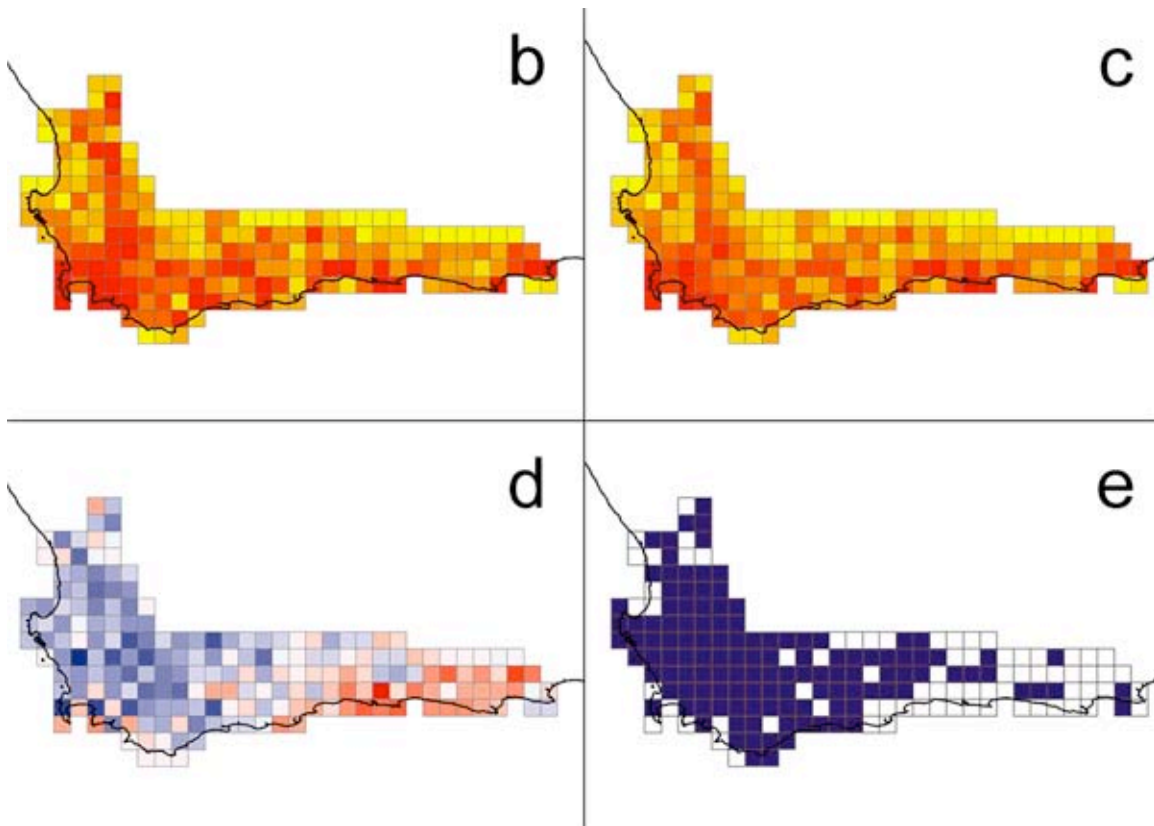
### Conservation

Taxonomic information has a core role to play in informing conservation strategy. Not only have the defects of taxon choices been demonstrated to play a very important part in determining the effects of a given action (Isaac *et al.* 2004), but when linked to phylogenetic or systematic information, taxonomic information allows a whole suite of novel and informative biodiversity metrics to be calculated, and when spatial data are linked to those taxon concepts a further range of powerful conservation area selection techniques can be employed. Both these goals are some of the most important potential uses of the toolbox.

#### ***Phylogenetic Diversity metrics (PD).***

Conventional conservation strategy, when it operates above the species level, tends to assume that all species are equal and that preserving the largest number of species is a goal in and of itself (Mace *et al.* 2003). Whilst this logic is fine when there is no need to prioritise amongst species, when resource pressures force prioritization then the assumption of equal worth may not be justified (McIntyre *et al.* 1992). Phylogenetic information provides one amongst

many ways of performing this prioritization (May 1990). The pair-wise distances amongst species in a phylogeny are a measure of the biological differences amongst them that have accumulated over the course of evolution (Faith 1994); there is clear analogy here with the underlying rationale for the use of PCMs (Owens and Bennett 2000). Put another way, the worth of a set of species is expressed not simply as their number, but as the sum of the accumulated genotypic and phenotypic divergence between them; the currency units of diversity: this is best expressed simply as the **sum of the branch lengths connecting the species on a phylogeny**. Consequently a set of three species of mouse would rate a much lower diversity score than did a set comprising a mouse, a sloth and a panda. This intuitively appealing and powerful metric is known as PD (phylogenetic diversity) (Faith 1992), although other related metrics exist (Vane-Wright *et al.* 1991).



**Figure 4: Compound figure from Forest *et al.* (2007) showing the highly congruent distribution of taxon richness (b) and PD (c) can obscure geographical pattern in the expected amount of PD for a given level of richness, as shown by residuals from a non-parametric regression (d; +ve = red, -ve = blue) and by randomisation tests (e; significantly low PD = blue)**

Recently, the use of PD metrics has been called into question (Rodrigues *et al.* 2005) because it was found through simulation that PD tends to scale in a predictable way with taxon richness. However, in a recent paper that utilised

some of the prototype functions from the toolbox (Forest *et al.* 2007), it was shown that even a close correspondence between taxon richness values and PD values can hide an important decoupling. That is: some areas have more or less PD than you would expect given their number of species (see Figure 4), and that this variation means that choosing areas to maximise the number of taxa protected does not protect the maximum possible PD. This decoupling of diversity metrics has some quite profound implications for conservation strategy should the tools to examine it be made more widely available.

### **Conservation Area Selection.**

Many problems in conservation biology fall into one of two main classes: a) how much resource is needed to achieve a goal? and b) given a level of resources what is the best goal to achieve? When applied to conservation area selection (Williams *et al.* 2005), these questions become:

- 1) **What is the smallest number of areas with which all species in a set can be protected (the *Species Set Covering Problem - SSCP*), and where are they?**
- 2) **What is the largest number of species that can be protected with  $n$  areas (the *Maximal Covering Species Problem - MCSP*), and which species and areas are they?**

Both these classes of problems are solved efficiently by a branch of mathematics known as “integer programming” (IP). In many cases, the solutions to these combinatorial problems are guaranteed to be optimal: although when there are many possible solutions it may not be practical to elucidate all (or even many) of them, for many conservation questions it is sufficient to know that one course of action is not sub-optimal.

Expressing the two questions as IP problems following Williams *et al.* (Williams *et al.* 2005), with a binary integer objective function to be minimized or maximised, and a set of linear constraints makes it apparent how solutions are found:

*The SSCP:*

$$\begin{aligned} & \text{Minimize } \sum_{j \in J} x_j \\ & \text{subject to } \sum_{j \in M_i} x_j \geq 1 \quad \forall i \in I \end{aligned}$$

where  $I$  is the set of all species  $i$ ,  $J$  is the set of all areas  $j$  inhabited by any  $i$  and  $M_i$  is the set of all  $j$  inhabited by  $i$ . The decision variable  $x_j$  is constrained to be binary, and the linear constraint forces at least one area from the range of each species to be chosen.

*The MCSP:*

$$\begin{aligned} &\text{Maximise} && \sum_{i \in I} y_i \\ &\text{subject to} && y_i \leq \sum_{j \in M_i} x_j \quad \forall i \in I \\ &\text{and} && \sum_{j \in J} x_j = P \end{aligned}$$

where the binary decision variable  $y_i$  takes 1 only if species  $i$  is chosen from amongst  $I$ , but where  $y_i$  can take 1 only if 1 or more of areas  $j$  from the range of  $i$  is chosen. This choice of area  $j$  is limited only by the single constraint that the sum of (binary) variable  $x_j$  is equal to the fixed number of areas  $P$  that represents available resources.

Alone, these powerful techniques would be a valuable addition to the toolbox. However, it is possible to integrate the MCSP with the PD metric in order to ask the question “how much PD can I protect with a given level of resources?”. By comparing this answer with that to the species equivalent question to find out if simply protecting as many species as possible is a suboptimal choice for a given situation. The translation to the PD case (Rodrigues and Gaston 2002) is accomplished by letting  $I$  represent the set of branches  $i$  in the phylogeny, and  $M_i$  to be the set of areas inhabited by any species descended from that branch, then solving by maximising  $y_i$  as before. Note that the solution to the PD equivalent of the SSCP is identical to the original SSCP, because to protect the entire tree one must protect every species.

## **The design of the toolbox**

We are now in a position to make a number of design requirements of the proposed toolbox. Firstly it must be network aware: incoming taxonomic, phylogenetic and geographic data sources are all accessed via the internet. Secondly, it must have a strong relational database core: not only does the taxonomic data require relational lookups in order to be able to implement handle multiple synonyms, but in addition several of the analytic functions (richness mapping, PD area selection) would benefit from easy access to a relational database. Third, users must be able to integrate these data into a number of complex simulation, mathematical and statistical analyses easily, and

produce graphical output that is immediately suitable for publication. Fourth, the user must be able to do all this without significant training in use, and via a standardised user interface. And finally, this should all be accomplished with minimum maintenance throughout the lifetime of the tool.

It would be possible to achieve all these goals with a single executable written in a low-level programming language such as C or its derivatives. There are several reasons why such an outcome may not be the optimal way to proceed:

- 1) **Redundant programming tasks.** Whilst there exist numerous code libraries for various biological data processing tasks in low-level languages (e.g. C or one of its descendants), there would necessarily be considerable programmer effort invested in combining and linking those routines together to perform a given toolbox function. For example, in order to demonstrate (using Pybus'  $\gamma$  statistic) a significant speciation rate change in a user's tree, the programmer would have to a) transfer information from a phylogenetic data format handler such as the NEXUS class library (Lewis 2003), to a binary tree data structure – see discussion in (Berry *et al.* 2005); b) write an algorithm to traverse that data structure correctly and calculate nodal depths; c) write an algorithmic implementation of the  $\gamma$ -statistic with reference to the original description (since the license allowing reuse of code from existing implementations is unclear (Pybus and Rambaut 2002) d) correctly calculate the null distribution of  $\gamma$  with reference to standard numerical techniques (Press *et al.* 1992) and f) return the statistic and associated p value with a portion of the user interface.

Whilst none of these steps is impossible, they are certainly time-consuming. In addition, they require that the initial programmer is both highly competent at the language of choice *and* a highly competent statistician and biologist, and that both statistical and biological expertise is available for support and error correction throughout the product's life.

- 2) **Time/feature-limited programming tasks.** There are simply too many components, each of them fully-featured and significant programming tasks in themselves, for the toolbox to be coded from scratch, or with reliance upon free class libraries in a compiled high-level language. Further, assuming the time and resources were available to code up a relational database for example, it is likely that the performance and feature set of the resulting component would be less than are currently available in pre-existing packages. This is particularly true of the GIS and scientific graphics components.
- 3) **OS-limited programming tasks.** Probably more than any other factor, the choice of target operating system has limited the take-up of taxonomic and phylogenetic software by the wider community. As a prime example, PAUP\*,

the tool of choice for the majority of systematists for the last decade, has been responsible for the population of many systematists offices with Apple hardware that is usually not present in the laboratories of their ecologist neighbours. Consequently the ecological audience has not been well served with phylogenetic programs suitable for the novice.

It might, therefore, be imagined that developing a multi-platform application, compiled against a number of major operating systems (Windows, OSX, Linux), would be the sensible solution. The existing software landscape indicates exactly how difficult this is to achieve in practice. In essence, only command-line tools such as MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) have reliably colonised all three environments. With the notable exception of TreeView (Page 1996), very few GUI (graphical user interface) programs have become ubiquitous because the developer effort of maintaining three separate code bases (due to the lack of a reliable cross-platform GUI kit) is prohibitive. And yet a user-friendly, consistent GUI is a prerequisite for the toolbox.

As a result of these considerations, we can state several features of the toolbox “design philosophy”:

- 1) Minimise novel coding by using existing code and software as much as possible.
- 2) The toolbox is not to be a single standalone program, but a suite of existing software tools, modified for biodiversity analysis.
- 3) Maintenance of the toolbox should, as much as possible, benefit from the IT expertise of the host institution, and not be contingent upon the biological or mathematical abilities of a single person.

Further, we can suggest some desiderata:

- 4) The toolbox should not be limited to a single operating system,
- 5) The toolbox should be as freely distributed as possible,

The sum of these requirements leads us to an interesting design decision. Since the best databases, GIS tools and analytical functions already exist as separate software packages, with their own user base, maintenance communities and distribution mechanisms, the EDIT toolbox will not be a piece of new software, but an integration of existing tools: this integration takes places at the operating system level, not at the executable level, and once some coding is done to allow the different software packages to communicate (Figure 5), maintenance of the toolkit becomes not a programming task but a systems administration task in which most organisations have considerable experience.

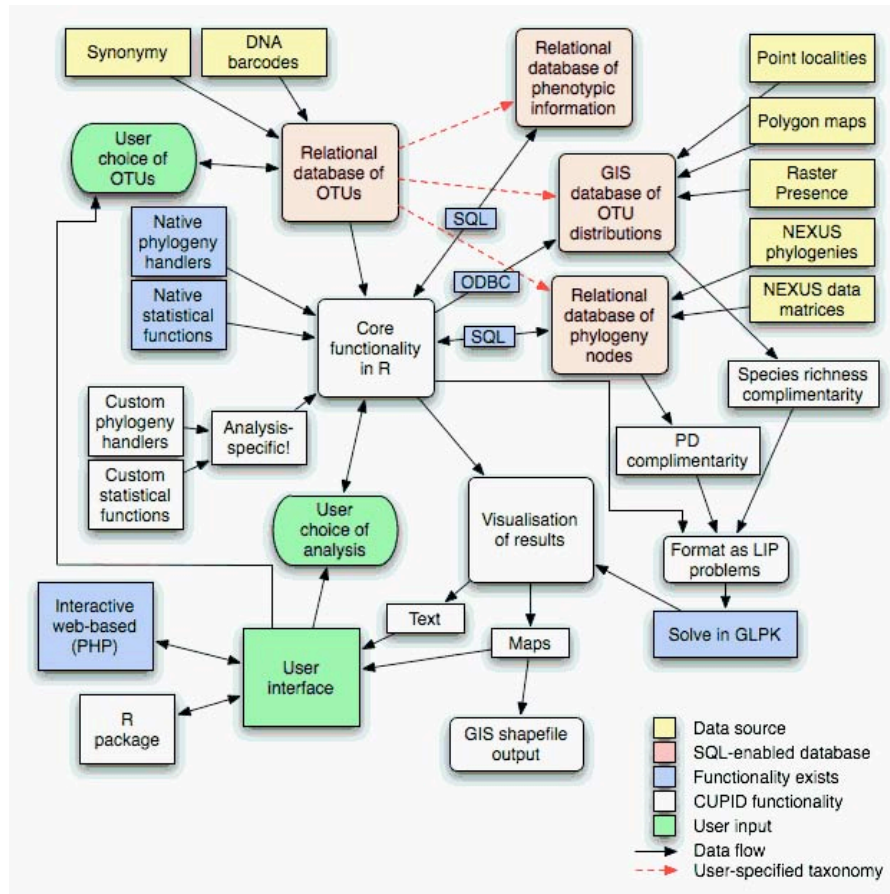
The EDIT toolbox is therefore best actualised as a Live LINUX distribution. To the layman, this means simply that it will be distributed as either a CD or preferably a USB stick. It will run on any current ix86 hardware setup (note this includes all recent Apple hardware) and self-configure to the local hardware. The toolbox itself will contain a lean operating system, which simultaneously maximises performance (even on older hardware) and ensures that local variations in available libraries, permissions or versions of operating systems do not prevent the toolbox from functioning. It is becoming quite commonplace to assemble entire linux-based operating systems for a particular purpose – for example the UK Natural Environment Research Council produce just such a live linux distribution, tailored for sequence bioinformatics use (BioLinux - <http://envgen.nox.ac.uk/biolinux.html>). Tools to tailor, make and configure USB-based linux distributions are available at <http://www.live-linux.org>.

Before moving onto precise design features, it is worth bearing mind what the structure of the toolbox will be. On top of the Linux core operating system, the toolbox will comprise the following GPL (i.e. freely available, modifiable and redistributable with attribution) licensed software:

a) ***The statistical and graphical environment R*** (<http://www.r-project.org>), which will handle all analysis, scientific functions, and graphical output. In addition, R provides interface functionality to GIS, database and system calls, so the “glue” code that gets the different components passing information between each other can be written in R. If convenient, however, it could be implemented as shell scripts. The final important R feature is obtained through an add-on package, R-PHP [<http://dssm.unipa.it/R-php/>]. R-PHP simply shifts the R input and output streams from the command line to a suitably configured webserver. The user issues R commands and receives R output (text and graphics) using a standard web browser. Further, R can be linked in to any web application written in PHP (an industry standard language), so that a full easy-to-use GUI for the toolbox can be written in PHP, and accessed via a web browser. Finally, R can issue SQL, SOAP, XML and Javascript queries through network sockets, so that queries of remote data sources can all be handled from within R itself.

b) ***The industry-leading relational database postgresql*** (<http://www.postgresql.org>), which will have a dual purpose. First it will underly the PHP web application that allows the toolbox components to be coordinated. Second it will be an analytic database in which users can place data downloaded from network resources, and that can be passed to or result from calculations in R. In addition, postgresql has a GIS-component, PostGIS ([www.postgis.org](http://www.postgis.org)), that adds support for geographic referencing and manipulation to database objects.

c) **The industry-standard webserver Apache ([www.apache.org](http://www.apache.org)) and application language PHP ([www.php.org](http://www.php.org))** which will run the PHP web application that interfaces R and postgresql. The user will interact with the toolbox by pointing their web browser at their own machine. This has the added advantage that the whole toolbox can be run on a central server for institutional use with multiple users. This would simply require an addition to the PHP code for user authentication and data storage; code libraries to do this are available under the GPL at a number of repositories.



**Figure 5: Design schematic for the toolbox. The working title of the proof-of-concept software is CUPID (“Kew PD”), hence white boxes represent “glue” code in R that needs to be written to achieve the design goals specified in this document, whereas blue code exists already as part of the various toolbox components and add-on packages. OTUs (operational taxonomic units) represent the taxa that the user has decided are ‘real’ for the purposes of a given analysis; as can be seen, the design of the toolbox reinforces to the user that taxonomic choices underlie the quantitative results of their analyses.**

## Network Data Sources

Note: an API is a generic term for the instructions by which a program (e.g. an online database or a software library) can be interacted with by a user or their program. Well-written databases and software have a published API distributed with them, so that third-parties can write their own software to interact correctly with the original program.

### Taxonomy

The toolbox has been designed to be non-prescriptive. It must be able, at the users instructions, to query remote data sources that are increasingly available to obtain data that would have been previously very time-consuming for the user to access. However, each of the network data inputs must have a user-specified analogue: if a user wants simply to input their own set of taxonomic choices, phylogenetic trees and/or distribution data, and simply use the toolbox as an analysis platform, that should be encouraged, whilst still allowing the user with none of the above data to access each of the data types through the internet.

In many ways, taxonomic data is the most difficult type still to access through the internet. Indeed, much of the EDIT programme is designed to facilitate this process. Consequently, it is difficult to design precise handlers for remote taxonomic data sources until the design of the EDIT outputs themselves is finalised. Several other data sources exist, however.

Firstly, and perhaps most importantly, is the CATE design ([www.cate-project.org](http://www.cate-project.org)) for taxon-specific taxonomic clearing-houses. A CATE node, once the design is finalised, will be able on interrogation via the internet to provide a consensus taxonomy for its group, together with the assenting and dissenting opinions that underlie the consensus. Clearly, this information is of the highest importance for the non-taxonomist who wishes simply to know which taxa the taxonomic community currently regards as real. Therefore, building an R handler in the toolbox to communicate with the CATE API considered a very high priority should the CATE model be adopted upon its final launch.

Secondly, various commercial taxonomic resources are being developed and might reach stability in the lifetime of the development process. Chief amongst these are the ZooBank project ([www.zoobank.org](http://www.zoobank.org)), ITIS (<http://www.itis.org>), IPNI (<http://www.ipni.org>), the Species2000 list (<http://www.sp2000.org>), and uBio (<http://www.ubio.org>). Whilst interrogating these lists via HTTP queries is simple to do, it should be noted that many of these resource yet has the synonymy information that really makes taxonomic data warehousing useful. It should therefore be noted that some development effort might need to be put into developing taxon-specific query agents for resources where this information

is available. One good example would be the Mammalian Species Of The World (<http://nmnhgoph.si.edu/msw/>) (Wilson and Reeder 2005) website and database, for which synonym information can be queried, and which will have a stable API and web address on its launch in mid-2007.

### Phylogeny

Again, many of the major phylogenetic resources on the internet are in a state of transition. Currently, the pre-eminent resource is TreeBASE (<http://www.treebase.org>), which is manually searchable but which does not have an API for agent use. A new revision of the existing database is planned that will have a direct SQL API, but it is not at present available and is behind schedule. Furthermore, a complete revision (TreeBASE II) is planned as part of the NSF CIPRES initiative, and will have a SOAP API which will be query-able from within R. At present, however, access to TreeBASE I will have to be through some of the “screen-scrape” HTML access functions that are present within the apTreeshape package in R (Bortolussi *et al.* 2006)

More immediate success can be had with the automated retrieval of sequence data from networked resources. R contains several add-on packages which facilitate the querying and downloading of tagged sequence data. Of particular note are the packages APE (Paradis *et al.* 2004), SEQINR (Charif and Lobry 2006) and the BioConductor suite (<http://www.bioconductor.org>). The R-package APE (Paradis *et al.* 2004) contains functions to estimate parameters in multiple models of molecular evolution, and to fit topologies via neighbour-joining algorithms. Topology-evaluation under ML and tree-searching should be available within APE within a year.

As a result of the generally poor availability at present of phylogenetic information over the internet, it is imperative that the toolbox be able to read and write standard (Newick, NEXUS) format phylogenetic trees that have been created in other more standard phylogenetic packages. Such functionality can already be found in the APE package. It is worth considering whether several of the GPL phylogenetic tools (for example PHYLIP (Felsenstein 2007) MrBayes (Huelsenbeck and Ronquist 2001) and r8s (Sanderson 1997)) should be included in the toolbox for ease of use.

### Geographic data

Increasingly large amounts of geographic data are available via internet resources, and with an underlying GIS database such as PostGIS, that can be operated through handler functions in R. Making use of this distributional information is a key feature of the toolbox. However, to begin, it is worth noting that R already has within it the ability to read and write ESRI format shapefiles

and ArcInfo coverages. Consequently, a facility for user to upload their own distributional data for analysis is an imperative. Likewise, once geographic analysis has been completed, the ability for the user to output results in one of these formats is also important.

The preminent resource for distributional data via the internet is the GBIF facility and the local nodes that distribute such information. However, there is still considerable fluidity (see, for example, <http://newportal.gbif.org>) in the APIs for accessing GBIF distributional data, and considerable effort will need to be expended to integrate the toolbox with this rapidly changing target.

One other notable geographic resource that deals with the biodiversity data for north and south America is NatureServe (<http://www.natureserve.org>). NatureServe is beginning to implement APIs for much of its data. The spatial data, however, is often hedged around with limitations on usage and which seriously undermine its utility as a biological resource. However, it is possible that at some time in the future realistic usage of the dataset will become possible and a close watch should be kept upon the development of the API ([http://services.natureserve.org/about/species\\_location\\_data.jsp](http://services.natureserve.org/about/species_location_data.jsp)) as a target.

Various local and continental atlas projects (the VLIZ datacentre – [http://www.vliz.be/EN/Data\\_Centre/Data\\_Centre\\_intro](http://www.vliz.be/EN/Data_Centre/Data_Centre_intro); the Atlas Flora Europaea project - <http://www.fmnh.helsinki.fi/english/botany/afe/index.htm>) have been digitized over the last few years; many of these data providers are involved in the Edit project in various forms, and considerable effort needs to be put into allowing the toolbox to interface with these data providers. Many of the datasets are already integrated into GBIF in various forms, but a direct API would ease and simplify the access process.

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