## Reading

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Nature Biotechnology 26, 303-304 (2008)
doi:10.1038/nbt0308-303
What is principal component analysis?
Markus Ringnér ${ }^{1}$
Principal component analysis is often incorporated into genome-wide expression studies, but what is it and how can it be used to explore high-dimensional data?

PCA as a tool to Quantify and Visualise
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What is the difference between these two pairwise correlation matrices?

|  | $\mathrm{X}_{1}$ | $\mathrm{X}_{2}$ | $\mathrm{X}_{3}$ | $\mathrm{X}_{4}$ | $\mathrm{X}_{5}$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{X}_{1}$ | 1.00 | 0.80 | 0.90 | 0.78 |
| $\mathrm{X}_{2}$ | 0.80 | 1.00 | 0.76 | 0.87 | 0.78 |
| $\mathrm{X}_{3}$ | 0.90 | 0.76 | 1.00 | 0.78 | 0.89 |
| $\mathrm{X}_{4}$ | 0.78 | 0.87 | 0.78 | 1.00 | 0.95 |
| $\mathrm{X}_{5}$ | 0.87 | 0.78 | 0.89 | 0.95 | 1.00 |
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|  | X ${ }_{1}$ | $\mathrm{X}_{2}$ | $X_{3}$ | X ${ }_{4}$ | $\mathrm{X}_{5}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{X}_{1}$ | 1.00 | 0.87 | 0.96 | 0.04 | 0.05 |
| $\mathrm{X}_{2}$ | 0.87 | 1.00 | 0.95 | 0.03 | 0.07 |
| $\mathrm{X}_{3}$ | 0.96 | 0.95 | 1.00 | 0.04 | 0.05 |
| $\mathrm{X}_{4}$ | 0.04 | 0.03 | 0.04 | 1.00 | 0.84 |
| $\mathrm{X}_{5}$ | 0.05 | 0.07 | 0.05 | 0.84 | 1.00 |

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## Ordination analyses

- Uncover, organize and summarize the main patterns of variation in a set of variables measured over multiple observations.
- Patterns of variation are structured in a reduced space with smaller number number of dimensions.
- Reduction is possible because often variables are associated (e.g., correlated). Dimensions represent combinations (e.g., linear combinations of variables).

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## Ordination analyses

A procedure for adapting a multidimensional swarm of data points in such a way that when it is projected onto a reduced number of dimensions any intrinsic pattern will become apparent.

Adapted from Connie Clark
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Ordination analyses - uncover and organize data; a quick example:

Species
Species


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## Ordination methods

- Principal Component Analysis (PCA)
- Correspondence Analysis (CA)
- Principal Coordinate Analysis (PCoA)
- Discriminant Function Analysis (DFA)
- Principal Curve Analysis
- Etc, etc, etc...

Principal components analysis (PCA) is perhaps the most common technique used to summarize patterns among variables in multivariate datasets.

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## Some treat Principal Component Analysis (PCA) as an unsupervised learning method (an exploratory technique such as k -means)



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Supervised versus unsupervised learning techniques $\qquad$

- Techniques for unsupervised learning are fast growing in a $\qquad$ number of fields, particularly biology.
- A cancer researcher might assay gene expression levels in 100 patients with breast cancer. They might then look for subgroups among the breast cancer samples, or among the genes, in order to obtain a better understanding of the disease.
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- A search engine might choose what search results to display to $\qquad$ a particular individual based on the click histories of other individuals with similar search patterns. These statistical learning tasks, and many more, can be performed via $\qquad$ unsupervised learning techniques.

Adapted from James et al. 2013

## Supervised versus unsupervised learning techniques

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In contrast, unsupervised learning is often much more challenging. The exercise tends to be more subjective, and there is no simple goal for the analysis, such as prediction of a response.

Unsupervised learning is often performed as part of an exploratory data analysis.

Hard to assess the results obtained given that there is no universally accepted mechanism for performing cross-validation or validating results on an independent data set; there is no way to check how the models does because we don't know the true answer-the problem is unsupervised.
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Adapted from James et al. 2013

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Principal components analysis (PCA) - example 1 $\qquad$
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A subtype of childhood acute lymphoblastic leukaemia with $\Rightarrow$ @ poor treatment outcome: a genome-wide classification study $\qquad$


Summary
Background Genetic subtypes of acute lymphoblastic leukemia (ALL) are used to determine isk and treatment in Lexetonod 2009: 10:12534
hildren 25\% of precussor B.AL cases are children. $25 \%$ of precursor B -ALL cases are genetically unclassified and have intermediate prognosis. We aimed to
use a genome.wide study to improve prognostic classification of ALL in children.

## Quantification and Visualisation

| A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study | ( ${ }^{\dagger}$ |
| :---: | :---: |
| Monique L Den Eoer', Majponvan Slegtenharst', Renve X De Menezes, Meyling H Chrok, Jessita G CA M Buis-Gladdnes, Susan TCJM Peters, Laura) CM Van Zutuen, H Eiema Beverloa, Peter /Van der Spek, Gaby Eschericht, MartinA Horstmann), Gritta E Janka-Schaubt, Willem AKamps $\ddagger$, Whian EEvans, Rob Pieters |  |
| Summary <br> Background Genetic subtypes of acute lymphoblastic leukaemia (ALL) are used to determine risk and treatment in children. $25 \%$ of precursor B-ALL cases are genetically unclassified and have intermediate prognosis. We aimed to use a genome-wide study to improve prognostic classification of ALL in children. | Pudinhedorline DO:1010165 524,0 |

Gene expression
(190 patients) $\square$
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Principal components analysis (PCA) - example 1 $\qquad$


Each letter is a patient.
$\qquad$ different lymphoblastic leukaemia (ALL) types.

Data matrix: 190 $\qquad$
observations by 22283 columns.

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Principal components analysis (PCA) - example 2 $\qquad$
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PCA - A Powerful Method for Analyze Ecological Niches $\qquad$
Franc Janžekovič and Tone Novak University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Maribor $\qquad$
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Principal components analysis (PCA) - example 2 $\qquad$
2.1 Environmental niche of three hymenopteran and two spider species

Between 1977 and 2004, 63 caves and artificial tunnels were ecologically investigated in
Slovenia; the three most abundant Hymenoptera species found in these studies have been ecologically evaluated (details in Novak et al. 2010a). In the caves, many environmental data were collected, as follows. The following abbreviations of the environmental variables are used: Dist-E = distance from entrance; Dist-S = distance from surface; Illum = illumination, CS temperature; $\mathrm{HY}=$ substrate moisture. The hymenopteran spatial niche breadth was original
represented by nine variables.

Data matrix: 63 observations (caves) by 9 columns
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Environmental variables (9) $\qquad$
63 caves $\square$

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Principal components analysis (PCA) - example 2 (pairwise correlation among environmental variables) $\qquad$

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Principal components analysis (PCA) - example 2 (niche differences - dots represent different caves ellipsoids are

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Fig. 5. Ordination of the nine environmental variables in $1^{\text {st }}$ and $2^{\text {nd }}$ PC axes. Ellipses ( $95 \%$ confidence) represent spatial niches in the three hymenopteran species.
interpretation variability in the data, essentially re-projecting the data on these coordinate system. As such, PCA represents associations among variables (gene, environmental variables) and data points are re projected so that the correlations among variables is maximize
$\bullet$ ©emae biids • male tirds
$26 \quad 28 \quad 30{ }^{38} \quad 32$
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Principal Component Analysis (PCA): A geometric interpretation
PCA finds the coordinate system (called principal components) that best represents the internal variability in the data, essentially re-projecting the data on these coordinate system. As such, PCA represents associations among variables (gene, environmental variables) and data points are re
$\qquad$ projected so that the correlations among variables is maximized
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Principal Component Analysis (PCA): A geometric interpretation

- PCA constructs a new coordinate system (new variables, PCs) which are linear combinations of the original data and which are defined to align the samples along their major axes of variation (assuming linearity).
- Thus, PCA determines the coordinate system that best represents the internal variability in the data, essentially re-projecting the data.

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The association among variables need to $\qquad$ be measured by either (in most cases):

Correlation Matrix (for variables that have different units or scales, e.g., ph, temperature). $\qquad$

Covariance Matrix (variables have the same $\qquad$ units, e.g., body length \& body width in cm).

Raw data when variables are in the same units
$\qquad$ (more difficult to interpret) and calculations differ (very rare to find applications in the literature); rarely used.

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\begin{aligned}
& \text { Correlation versus covariance } \\
& \begin{array}{r}
\operatorname{COV}_{x y}=\frac{\sum_{i=1}^{n}\left(X_{i}-\bar{X}\right)\left(Y_{i}-\bar{Y}\right)}{n-1} \\
X=0 \& Y=0 \therefore s_{x}=s_{x} \& s_{y}=s_{y} \\
C O R_{x y}=\frac{C O V_{x y}}{s_{x} s_{y}} \\
X=0 \& Y=0 \therefore s_{x}=1 \& s_{y}=1
\end{array}
\end{aligned}
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The mathematics of Principal Component Analysis (PCA):
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Eigen-analysis is a mathematical operation on a $\qquad$ square symmetric matrix (e.g., pairwise correlation matrix, pairwise covariance matrix).
A square matrix has the same number of rows as columns.

A symmetric matrix is the same if you switch rows and columns. $\qquad$
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square and symmetric matrix $\qquad$
(e.g., pairwise correlation matrix) $\qquad$

|  | $\mathrm{X}_{1}$ | $\mathrm{X}_{2}$ | $\mathrm{X}_{3}$ | $\mathrm{X}_{4}$ | $\mathrm{X}_{5}$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | 1.00 | 0.80 | 0.90 | 0.78 | 0.87 |
| $\mathrm{X}_{1}$ | 1.80 |  |  |  |  |
| $\mathrm{X}_{2}$ | 0.80 | 1.00 | 0.76 | 0.87 | 0.78 |
| $\mathrm{X}_{3}$ | 0.90 | 0.76 | 1.00 | 0.78 | 0.89 |
| $\mathrm{X}_{4}$ | 0.78 | 0.87 | 0.78 | 1.00 | 0.95 |
| $\mathrm{X}_{5}$ | 0.87 | 0.78 | 0.89 | 0.95 | 1.00 |
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The important components of Principal Component Analysis (pun intended)

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Principal component analysis presents three important structures:

1 - Eigenvalues: represent the amount of variation in the original data summarized by each principal component. The first principal component (PC-1) presents the largest amount, PC-2 presents the second largest
$\qquad$ amount, and so on.

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Eigenvalues:

| PC | eigenvalues | $\%$ |  |
| :---: | :---: | :---: | :--- |
| 1 | 2.867 | 0.573 | "Higher" dimensionality |
| 2 | 1.827 | 0.365 |  |
| 3 | 0.167 | 0.033 |  |

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Principal component analysis presents three important structures:

2 - Eigenvectors: Each principal component is a linear function with coefficients for each variable.

- Eigenvectors contain these coefficients. High values, positive or negative, represents high association with the component.
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Eigenvectors can be seen as regression coefficients, where the component is the dependent variable. A "one dimension" matrix has only one interpretable principal component.

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\begin{aligned}
& \text { PC-1=0.447X }{ }_{1}+0.432 X_{2}+0.445 X_{3}+0.450 X_{4}+0.462 X_{5} \\
& \text { Unlike the numbers after }=\text {, this is not a subtraction but a } \\
& \text { hyphen stating that this is the first and second Principal Components (PC). }
\end{aligned}
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| $\mathrm{X}_{1}$ | X 2 | X ${ }_{3}$ | $\mathrm{X}_{4}$ | $\mathrm{X}_{5}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| ${ }^{1} 1$ | 0.87 | 0.96 | 0.04 | 0.05 | "two |
| $\mathrm{x}_{2}$ 0.87 <br> $x^{\prime}$  <br> l  | 1.00 | 0.95 | 0.03 | 0.07 | dimensions" |
| $\mathrm{X}_{3}$ 0.96 <br> $\mathrm{X}_{4}$ 0.04 | 0.95 0.03 | 1.00 <br> 0.04 | 0.04 1.00 | 0.05 0.84 | dimensions |
| K54 | 0.07 | 0.05 | 0.84 | 1.00 |  |

Associated eigenvectors (only interpret the first two components (PC)

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Principal component analysis presents three important structures:

3 - Multivariate scores: Since each component is a linear function of the variables, when multiplying the standardized variables (in the case of correlation matrices) by the eigenvector structure, a matrix containing the position of each observation in each principal component is produced.

The plot of these scores in the first few dimensions, represents the main patterns of variation among the original observations (more in the empirical example).

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\begin{aligned}
& \text { PC-1 }=0.569 X_{1}+0.567 X_{2}+0.585 X_{3}+0.072 X_{4}+0.085 X_{5} \quad \underset{1}{\text { var }} \underset{1}{1.569-0.064} \\
& \text { PC-2 }=-0.064 X_{1}-0.060 X_{2}-0.067 X_{3}+0.704 X_{4}+0.702 X_{5} \quad 2{ }_{3}^{2} \\
& \begin{array}{l|ll}
3 & 0.585 & -0.067 \\
4 & 0.072 & 0.704 \\
\hline
\end{array} \\
& \begin{array}{l|l|l|l}
5 & 0.085 & 0.702 \\
\hline
\end{array}
\end{aligned}
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Next lecture: How many PCA dimensions? Inferential frameworks for determining number of axes to interpret and the significance of each variable on each axis (lots of work on this area).
$1^{\text {st }}$ ) determine how many axes to interpret (i.e., how many PCs capture correlated variation in the data?).


How many principal components? stopping rules for determining the number of non-trivial axes revisited Pedro R. Peres-Neto*, Donald A. Jackson, Keith M. Somers

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Inferential frameworks for determining number of axes to interpret and the significance of each variable on each axis are usually nor performed.
$\left.2^{\text {nd }}\right)$ for each significant axis, determine which variable is significant on each of them.
${ }^{\text {End }}$

GIVING MEANINGFUL INTERPRETATION TO ORDINATION AXES
ASSESSING LOADING SIGNIFICANCE IN PRINCIPAL COMPONENT ANALYSIS

Pedro R. Peres-Neto, ${ }^{1}$ Donald A. Jackson, and Keith M. Somers
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What is the correlation structure and differences among streams in terms of their environmental features?

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Depth
Depth variation
Current velocity
Current variation
Substrate composition: Boulder, rubble, gravel and sand
Substrate variation (variance in composition)
Stream width variation (irregularity)
Area
Altitude
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f site suitabilit,occurence of ishis secies in streams: the role
of site suitability, morphology and phylogeny versus species
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Correlation matrix
1.00}0.04 0.28 -0.07 0.06 -0.33 -0.02 0.12 -0.02 0.05 0.01 -0.11
0.04
0.28
-0.07
0.06
-0.33
-0.02
0.12
-0.02
0.05
0.01
-0.11
```

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Eigenvector structure (2 first dimensions) $\qquad$
PC-1 PC-2
depth $0.098416371-0.55557259$
DepthVar $-0.383072589-0.26772556$
velocity $0.145820452-0.22434910$
VelVar -0.409585483-0.15169873
boulder $-0.363399847-0.20189977$
rublle $\quad-0.204526467 \quad 0.50098773$
gravel $0.007091107 \quad 0.08935752$
sand $\quad 0.426264131-0.09866678$
altitude - $0.421467330-0.23396335$
$\begin{array}{llll}\text { area } & 0.229031867 & -0.02477526\end{array}$
irreg $\quad-0.165951470 \quad 0.09149688$
SediVar $-0.203159109 \quad 0.41607768$

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Eigenvector plot:


Component 1 variance: 30.87\%, $\mathrm{p}=0.01$

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Eigenvector plot :

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$\qquad$for the variables are beyond the confidence
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1) Latitude (Lat) and Longitude (Long) at the centre of geographic cell.
2) Average precipitation (last 40 years; avg_prec)
3) Average actual evapotranspiration (avg_ET, a proxy of productivity)
4) Average vegetation index (avg_VI)
5) Mean altitude (avg_Alt)
6) Maximum altitude minus minimum altitude (altitudinal range; range_Alt)
7) Average temperature (avg_temp)
8) Seasonal temperature (annual range in temperature; seas_temp)
9) Seasonal precipitation (annual range in precipitation; seas_prec)

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