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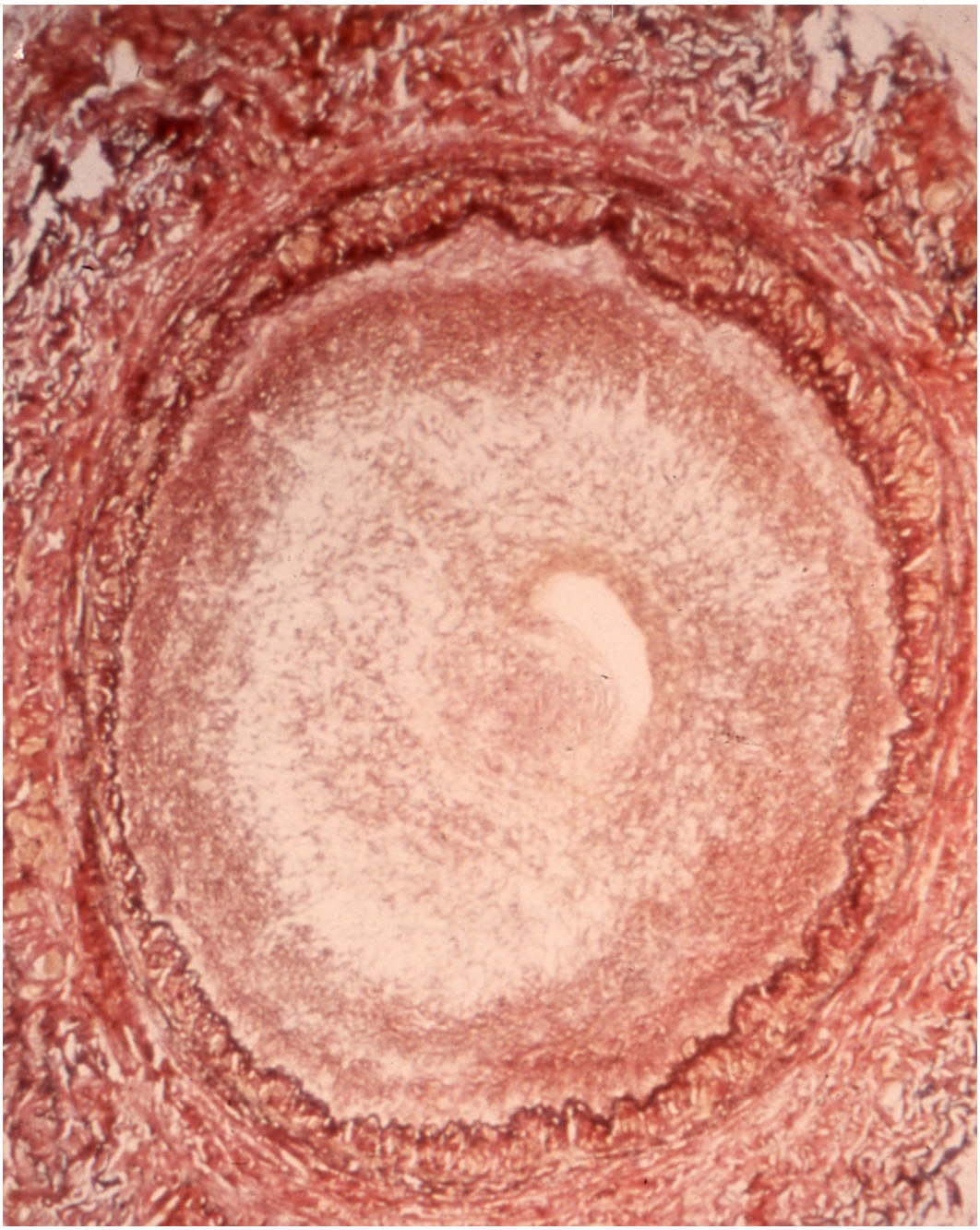

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The University of Malta Research, Innovation & Development Trust

Malta Chamber of Scientists

The Journal of the Malta Chamber of Scientists

Editor-in-Chief: Giuseppe Di Giovanni

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Chronological List of Past and Present Editors of Xjenza The Journal of the Malta Chamber of Scientists

2013–
Editor: Giuseppe Di Giovanni
Associate Editors: *David Magri, Ian Thornton, Ian Cassar, Philip Farrugia, Sebastiano D'Amico, Nicholas Sammut, David Mifsud, Godfrey Baldacchino, Liberato Camilleri, Carmel Cefai*
Xjenza Online Vol. 4 Iss. 2 - December 2016
Xjenza Online Vol. 4 Iss. 1 - July 2016
Xjenza Online Vol. 3 Iss. 2 - December 2015
Associate Editors: *David Magri, Ian Thornton, Ian Cassar, Philip Farrugia, Sebastiano D'Amico, Nicholas Sammut, Joseph Galea, David Mifsud, Sandro Lanfranco, Mario Valentino, Godfrey Baldacchino, Liberato Camilleri*
Xjenza Online Vol. 3 Iss. 1 - August 2015
Xjenza Online Vol. 2 Iss. 2 - October 2014
Xjenza Online Vol. 2 Iss. 1 - March 2014
Xjenza Online Vol. 1 Iss. 2 - October 2013
Xjenza Online Vol. 1 Iss. 1 - March 2013

2003–2007
Editors: Joseph N. Grima and Richard Muscat
Xjenza Vol. 12 - 2007
Xjenza Vol. 11 - 2006
Xjenza Vol. 10 - 2005
Xjenza Vol. 9 - 2004
Xjenza Vol. 8 - 2003

1996–2002
Editor: Angela Xuereb
Associate Editor: *Richard Muscat*
Xjenza Vol. 7 - 2002
Xjenza Vol. 6 - 2001
Associate Editors: *Martin Ebejer and Richard Muscat*
Xjenza Vol. 5 - 2000
Xjenza Vol. 4 Iss. 2 - 1999
Xjenza Vol. 4 Iss. 1 - 1999
Associate Editors: *Martin Ebejer, Richard Muscat, and Christian A. Scerri*
Xjenza Vol. 3 Iss. 2 - 1998
Xjenza Vol. 3 Iss. 1 - 1998
Associate Editors: *Martin Ebejer, Richard Muscat, Christian A. Scerri and Emmanuel Sinagra*
Xjenza Vol. 2 Iss. 2 - 1997
Xjenza Vol. 2 Iss. 1 - 1997
Xjenza Vol. 1 Iss. 2 - 1996
Xjenza Vol. 1 Iss. 1 - 1996

Scope of Journal

Xjenza is the Journal of the Malta Chamber of Scientists and is published in an electronic format. Xjenza is a peer-reviewed, open access international journal. The scope of the journal encompasses research articles, original research reports, reviews, short communications and scientific commentaries in the fields of: mathematics, statistics, geology, engineering, computer science, social sciences, natural and earth sciences, technological sciences, linguistics, industrial, nanotechnology, biology, chemistry, physics, zoology, medical studies, electronics and all other applied and theoretical aspect of science.

The first issue of the journal was published in 1996 and the last (No. 12) in 2007. The new editorial board has been formed with internationally recognised scientists, we are planning to restart publication of Xjenza, with two issues being produced every year. One of the aims of Xjenza, besides highlighting the exciting research being performed nationally and internationally by Maltese scholars, is to provide insight to a wide scope of potential authors, including students and young researchers, into scientific publishing in a peer-reviewed environment.

Instructions for Authors

Xjenza is the journal of the Malta Chamber of Scientists and is published by the Chamber in electronic format on the website: http://www.mcs.org.mt/index.php/xjenza. Xjenza will consider manuscripts for publication on a wide variety of scientific topics in the following categories

1. Communications
2. Research Articles
3. Research Reports
4. Reviews
5. Notes
6. News and Views
7. Autobiography

Communications are short peer-reviewed research articles (limited to three journal pages) that describe new important results meriting urgent publication. These are often followed by a full Research Article.

Research Articles form the main category of scientific papers submitted to Xjenza. The same standards of scientific content and quality that applies to Communications also apply to Research Articles.

Research Reports are extended reports describing research of interest to a wide scientific audience characteristic of Xjenza. Please contact the editor to discuss the suitability of topics for Research Reports.

Review Articles describe work of interest to the wide readership characteristic of Xjenza. They should provide an in-depth understanding of significant topics in the sciences and a critical discussion of the existing state of knowledge on a topic based on primary literature. Review Articles should not normally exceed 6000 words.Authors are strongly advised to contact the Editorial Board before writing a Review.

Notes are fully referenced, peer-reviewed short articles limited to three journal pages that describe new theories, concepts and developments made by the authors in any branch of science and technology. Notes need not contain results from experimental or simulation work.

News and Views: The News section provides a space for articles up to three journal pages in length describing leading developments in any field of science and technology or for reporting items such as conference reports. The Editor reserves the right to modify or reject articles for consideration as 'news items'.

Commentaries: Upon Editor's invitation, commentaries discuss a paper published in a specific issue and should set the problems addressed by the paper in the wider context of the field. Proposals for Commentaries may be submitted; however, in this case authors should only send an outline of the proposed paper for initial consideration. The contents of the commentaries should follow the following set of rules: 3000 words maximum, title 20 words maximum, references 10 maximum (including the article discussed) and figures/tables 2 maximum.

Errata: Xjenza also publishes errata, in which authors correct significant errors of substance in their published manuscripts. The title should read: Erratum: "Original title" by ***, Xjenza, vol. *** (year). Errata should be short and consistent for clarity.

Invited Articles and Special Issues: Xjenza regularly publishes Invited Articles and Special Issues that consist of articles written on invitation by the Editor or member of the editorial board.

Submission of Manuscripts

Manuscripts should be sent according to the guidelines given hereafter to submissionxjenzaonline@gmail.com.

Referees All manuscripts submitted to Xjenza are peer reviewed. Authors are requested to submit with their manuscript the names and addresses of three referees, preferably from overseas. Every effort will be made to use the recommended reviewers; however the editor reserves the right to also consult other competent reviewers.

Conflict of Interest Authors are expected to disclose any commercial or other associations that could pose a conflict of interest in connection with the submitted manuscript. All funding sources supporting the work, and institutional or corporate affiliations of the authors, should be acknowledged on the title page or at the end of the article.

Policy and Ethics The work described in the submitted manuscript must have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (`http://www.wma.net/en/30publications/10policies/b3/index.html`); EU Directive 2010/63/EU for animal experiments (`http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm`); Uniform Requirements for manuscripts submitted to Biomedical journals (`http://www.icmje.org`). This must be stated at an appropriate point in the article.

Submission, Declaration and Verification Submission of a manuscript implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that it has been approved for publication by all authors, and tacitly or explicitly, by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright-holder.

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Preparation of Manuscripts

Xjenza accepts submissions in MS Word, Libre Office Writer and LaTeX with the latter being the preferred option. Anyone submitting in LaTeX should use the journal template, the latest version of which can be found at `http://github.com/hicklin/Xjenza-Journal-Template`. All the necessary files to run the LaTeX document should be supplied together with the rendered PDF.

If a word processor is used the styling should be kept to a minimum, only introducing bold face, italics, subscript and superscript text where the context requires it. Text should be in single-column format and the word processor options should not be used in order to justify text or hyphenate words. Together with the native format of the word processor, a pdf, generated by the word processor, must be given. Furthermore, artwork should be in accordance to the artwork guidelines give below and must be submitted separately from the word processor file. Similarly, the bibliographic data of the cited material should be submitted separately as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTeX (*.bib) file.

Article Structure

A manuscript for publication in Xjenza will ordinarily consist of the following: Title page with contact information, Abstract, Highlights, Keywords, Abbreviations, Introduction, Materials and Methods, Results, Discussion, Conclusion, Appendices and References.

The manuscript will be divided into clearly defined numbered sections. Each numbered subsection should be given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by the section number as opposed to simply 'the text'.

Title page

- Title should be concise yet informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after each author's name and in front of the appropriate address. Provide full postal address of each affiliation, including the country name and, if available, the e-mail address.
- Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, including post-publication. Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and complete postal address. Contact details must be kept up to date by the corresponding author.
- Present/permanent address. If an author has changed the address since the work described, this can be indicated as a footnote to the author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract A concise and factual abstract is required of up to about 250 words. The abstract should state briefly the background and purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references and non-standard abbreviations should be avoided. If essential, these must be defined at first mention in the abstract itself.

Abbreviations Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention as well as in the footnote and should be used consistenly throughout the text.

Introduction State the objectives of the work and provide an adequate background, avoid a detailed literature survey or a summary of the results.

Materials and Methods Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results Results should be clear and concise. Numbered/tabulated information and/or figures should also be included.

Discussion This should explore the significance of the results of the work, yet not repeat them. Avoid extensive citations and discussion of published literature. A combined section of Results and Discussion is often appropriate.

Conclusion The main conclusions based on results of the study may be presented in a short Conclusions section. This may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Acknowledgements Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided assistance during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. Anyone using LaTeX should use the package siunitx in all cases.

Footnotes Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes by a superscript number in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table Footnotes Indicate each footnote in a table with a superscript lower case letter.

Artwork Electronic artwork General points:

- Make sure you use uniform lettering and sizing of your original artwork.
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- Number the illustrations according to their sequence in the text.
- Name your artwork files as 'fig$x$' or 'tab$x$' where x corresponds to the sequence number in your document.
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Formats Regardless of the application used, when your electronic artwork is finalised its file format should be one of the following (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

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Tables Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. Large tables should be submitted in CSV format.

Citations and References Reference and citation styles for manuscripts submitted to Xjenza should be in accordance to the APA v6 style.

Citation in text References to cited literature in the text should be given in the form of an author's surname and the year of publication of the paper with the addition of a letter for references to several publications of the author in the same year. For further information regarding multiple authors consult the APA v6 guidelines. Citations may be made directly

Kramer et al. (2010) have recently shown ...

or parenthetically

as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999).

Groups of references should be listed first alphabetically, then chronologically. When writing in LaTeX use `\textcite{}` and `\parencite{}` for the respective cases mentioned.

The reference section Every reference cited in the text should also be present in the reference list (and vice versa). The reference list should also be supplied as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTeX (*.bib) file. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication. Consult the APA v6 guidelines for multiple authors. Below are some examples of referencing different bibliographic material.

Reference to a Journal Publication:

Agree, E. M. and Freedman, V. A. (2011). A Quality-of-Life Scale for Assistive Technology: Results of a Pilot Study of Aging and Technology. *Phys. Ther.*, 91(12):1780–1788.

McCreadie, C. and Tinker, A. (2005). The acceptability of assistive technology to older people. *Ageing Soc.*, 25(1):91–110.

Reference to a Book:

Brownsell, B. (2003). *Assistive Technology and Telecare: Forging Solutions for Independent Living.* Policy Press, Bristol.

Fisk, M. J. (2003). *Social Alarms to Telecare: Older People's Services in Transition.* Policy Press, Bristol, 1st edition.

Reference to a Chapter in an Edited Book:

Brownsell, S. and Bradley, D. (2003). New Generations of Telecare Equipment. In *Assist. Technol. Telecare Forg. Solut. Indep. Living*, pages 39–50.

Web references The full URL should be given together with the date the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately or can be included in the reference list.

References in a Special Issue Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Journal Abbreviations Journal names should be abbreviated according to:

-Index Medicus journal abbreviations: `http://www.nlm.nih.gov/tsd/serials/lji.html`;

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-CAS (Chemical Abstracts Service): `http://www.cas.org/sent.html`.

Video data Xjenza accepts video material and animation sequences to support and enhance the presentation of the scientific research. Authors who have video or animation files that they wish to submit with their article should send them as a separate file. Reference to the video material should be clearly made in text. This will the modified into a linked to the paper's supplementary information page. All submitted files should be properly labelled so that they directly relate to the video files content. This should be within a maximum size of 50 MB.

Submission check list

The following list will be useful during the final checking of a manuscript prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

- One author has been designated as the corresponding author with contact details:
  - E-mail address.
  - Full postal address.
  - Telephone and fax numbers.
- All necessary files have been sent, and contain:
  - All figures are given separately in PDF, SVG, JPEG of PNG format.
  - Caption for figures is included at the end of the text.
  - All tables (including title, description, footnotes) are included in the text and large tables have been given separately as CSV.
  - The reference list has been given in XML, RIS, zotero.splite or BIB file format.
- Further considerations
  - Abstract does not exceed about 250 words.
  - Manuscript has been 'spell-checked' and 'grammar-checked'.

- References are in the required format.
- All references mentioned in the reference list are cited in the text, and vice versa.
- Bibliographic data for all cited material has been given.
- Permission has been obtained for use of copyrighted material from other sources (including the Web).
- A PDF document generated from the word processor used is given.

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Use of the Digital Object Identifier The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

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Di Giovanni, G. (2016). *Xjenza Online*, 4:97–99.

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Editorial

Twenty Years of Xjenza: Celebration of a Long Journey (1996–2016)

Giuseppe Di Giovanni[*1]
[1]*Department of Physiology and Biochemistry, University of Malta, Msida, Malta*

This year, Xjenza Online, one of the first Maltese journals covering all the branches of science, celebrates its twentieth anniversary. During the past two decades - since the first issues of artisanal workmanship until now - the journal has gone through many phases, has overcome challenges, has undergone changes in format and editorial policy, but it has remained true to its original purpose of being a prestigious means of dissemination of studies on science and, in particular, a critical observatory of the Maltese scientific reality.

The celebration of this important anniversary invites reflection and thus inescapable findings. In this regard, the readers find reproduced here pertinent speeches given by Dr Jeffrey Pullicino Orlando, CEO of MCST, Mr Kevin J. Ellul, University of Malta Director Library Services, University of Malta and Professor Alfred J. Vella, Rector of the University of Malta, on the occasion of the Celebration of *Xjenza 20* on the 23rd of September 2016 at the Aula Magna, University of Malta, Valletta Campus. These speeches highlight the importance of Xjenza and scientific publications in Science and Technology for national development (Figs. 1, 2, 3).

Figure 1: From the left, Rector Professor Alfred J. Vella, President of the Malta Chamber of Scientists Professor Alex Felice and E-i-C of Xjenza Professor Giuseppe Di Giovanni, at Celebrating Xjenza 20th Anniversary ceremony Aula Magna, Old University in Valletta on the 23rd of September 2016.

Figure 2: From the left, Joseph Grima (Xjenza Editor 2003–2007), current associate editors Joseph Galea (Medical Sciences), Liberato Camilleri (Mathematical and Statistical Science), Angela A. Xuereb Anastasi (Xjenza Editor 1996–2003), current associate editors Ian Thornton (Cognitive and Social Sciences), Giuseppe Di Giovanni (current Xjenza Editor), Philip Farrugia (Engineering Science), David Magri (Physics and Chemical Science), Ian Cassar (Economics), Mauro Pessia (Editorial Board member) at the Celebrating Xjenza 20th Anniversary ceremony on the 23rd of September 2016, Valletta.

The Xjenza team also includes: Sebastiano D'Amico (Geoscieces), David Mifsud (Life Science), Nicholas Sammut (Information and Communication Technologies), Carmel Cefai (Psychological Science), Godfrey Baldacchino (Social Sciences), the Copy Editor Jackson Said, the Editorial Assistants Katie Haywood and Amber Crews-Rees, Copy Editor Gabriel Farrugia and the Web Administrator John Gabarretta.

It must be recognized that in the past twenty years Xjenza has made significant efforts to achieve genuine internationalization. With this, we mean that, on the one hand, since its inception, Xjenza has stood out for the strong presence of international authors in its editions, not only in its editorial committee, but also in the authorship of articles or participation in interviews. On the other hand, in recent years, it has undertaken numerous efforts to ensure that Maltese research and production is disseminated and recognized. This is our commitment to the academic community, and our peers.

**Correspondence to*: Giuseppe Di Giovanni (giuseppe.digiovanni@um.edu.mt)

Figure 3: From the left, E-i-C of Xjenza Professor Giuseppe Di Giovanni and President of the Malta Chamber of Scientists Professor Alex Felice at Celebrating Xjenza 20th Anniversary ceremony on the 23rd of September 2016, Valletta.

This time, to mark the twentieth anniversary of Xjenza, its editorial board has planned this second commemorative issue of volume 4, which clearly expresses its desire for internationalization.

Moreover, in this volume, we present 3 commentaries on various hot issues in science. This is a new type of article that we have introduced in this issue, under editor invitation. The authors of these commentaries have in-depth knowledge of the topic and are eager to present a new and/or unique viewpoint on existing problems, fundamental concepts, or prevalent notions, or want to discuss the implications of a newly implemented innovation. We hope, in this way, to advance the research field by providing a forum for varying perspectives on a certain topic under consideration in the journal. The first of them, brings together 4 researchers from the Institute of Space Sciences and Astronomy, outlining the research projects of the Gravity Research Group at the University of Malta. The second commentary, written by Dr Roberto Frau, a new member of our editorial board from Italy, focuses on a new treatment for Tourette syndrome. Over the years, Frau and colleagues have demonstrated that the pharmacological inhibition, by drugs such as finasteride, of rate-limiting enzyme in androgens and neurosteroids synthesis, 5-alpha reductase, elicits therapeutic effects in animal models of TS. Unfortunately, the clinical applications of this drug on Tourette syndrome therapy remain limited; in fact, finasteride cannot be prescribed in children, who represent the broadest target population in this disorder; moreover it has been shown to possibly induce depression and reduction of libido. Dr Frau, for these reasons, stresses the importance of identification of the neurobiological bases and molecular mechanisms underlying the effects of finasteride and other similar drugs to overcome their limitations. The last commentary is authored by Dr Maurizio Casarrubea, another Italian member of our editorial team, on critical review of anxiety and anxiety-related behaviour analysis and the importance of T-pattern analysis, as a new technique able to evaluate the temporal characteristics of sequences of events of behaviours.

This issue includes three research articles. Liberato and colleagues recommend using the strengths and difficulties questionnaire (SDQ), proposed by Goodman in 1997, to measure social, emotional and behaviour, to adopt i) a multilevel structural equation model that accommodates both the hierarchical nested structure of the data, but also caters for the latent factor structure in the data and ii) to include student, classroom, school and home predictors in the model. The article by Carveni and colleagues from Italy describes the geological, geomorphological and archaeological sites in Pachino and Portopalo di Capo Passero areas in Sicily. Finally, Mifsud et al., focus their research on the flora of Selmunett (St. Paul's Island) including mosses and lichens. These are examined critically, identifying possible misidentifications as well as establishing their status, thus producing a final update of the florula of Selmunett.

Two interesting reviews on biomedical subjects complete the issue. The front cover is dedicated to the very interesting review written by Joseph Galea on the pathobiology of Saphenous vein grafts; the commonest conduit used for coronary artery bypass surgery, and the second by Bonetta and colleagues on protein structure-based approaches to drug discovery.

This second issue of the Jubilee volume (4) ends as usual with the News section. The last six months of 2016 have been very rich in scientific events in which Xjenza has played a major role. First, you will find the talks given at the Celebration of *Xjenza 20* by eminent speakers, followed by a piece by David Magri, the curator of Science in the House, organised by the Malta Chamber of Scientists, the University Research Trust (RIDT) and the Science in the City/European Researchers' Night consortium. Science in the City, European Researchers' Night is funded by the EU Marie Sklodowska-Curie Action of the Horizon 2020 (H2020) Programme.

We have continued the traditional and important aim of Xjenza Online to offer training in the art of scientific publishing in a peer-reviewed environment for young and more experienced researchers. With this aim, Xjenza, with ELSEVIER and the Library of University of Malt, was involved in the organization of a workshop on how to publish on the 23rd of October. This was very successful and drew in significant interest from the general public. Indeed, this will be repeated next year during the 6th Mediterranean Neuroscience Con-

ference that the Malta Chamber of Scientists and the Malta Neuroscience Network is organizing for the Mediterranean Neuroscience Society in June 12^{th}–15^{th}, 2017 at the Radisson in St Julian's. For Xjenza Online and neuroscience in Malta, this will be a fantastic showcase, indeed Xjenza Online will have a Special Issue for the Proceedings of the conference and a print copy will be distributed to all the attendees.

Publishing this special issue was the way in which the editorial board has chosen to celebrate the anniversary of Xjenza Online together with readers. And, finally, we expect this issue to fully demonstrate our conviction that scientific journals are important sources of information for teaching and research activities, and thus constitute an input for research and the development of science. On this anniversary, besides celebrating the maturity of Xjenza, we would like to congratulate and thank all those who daily make great efforts to ensure this important journal continues to exist.

Giuseppe Di Giovanni
Editor in Chief

Azzopardi, L. M., Camilleri, L., Sammut, F., and Cefai, C. (2016). *Xjenza Online*, 4:100–108.

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Research Article

Examining the Model Structure of the Strengths and Difficulties Questionnaire (SDQ)

Lara Marie Azzopardi[1], **Liberato Camilleri**[*1], **Fiona Sammut**[1] **and Carmel Cefai**[2]
[1]*Department of Statistics and Operations Research, University of Malta*
[2]*Department of Psychology, University of Malta*

Abstract. The Strengths and Difficulties questionnaire (SDQ), proposed by R. Goodman (1997), has been used by researchers to measure social, emotional and behaviour difficulties in children. The SDQ includes four difficulty subscales, measuring emotional, conduct, hyperactivity and peer problems. It also includes a fifth subscale, measuring prosocial behaviour. Dickey and Blumberg (2004) suggested that the SDQ factor structure can be reduced to three dimensions comprising the prosocial, externalisation and internalisation subscales. Externalising problems combine conduct and hyperactivity, while internalising problems combine peer and emotional difficulties. A sample of 5200 local students aged between 4 and 16 years was used to investigate the factor structure underlying the teachers' version of the SDQ. Statistical analysis was conducted using Exploratory Factor Analysis (EFA), Confirmatory Factor Analysis (CFA) and Structural Equation Modeling (SEM). The study finds that the three-factor solution fits the data well. EFA establishes good internal consistency of these three factors. Moreover, several fit indices confirm this three-factor model through CFA. The externalisation construct linking hyperactivity and conduct problems is more robust than the internalisation construct linking emotional to peer problems. Through SEM, it was deduced that the Externalisation Factor dominates both the Internalisation and the Prosocial Factors. This implies that by controlling externalized behaviour leads to a better control of internalized and prosocial behaviours of students.

Keywords: Social emotional and behaviour difficulties (SEBD), Exploratory Factor Analysis, Confirmatory Factor Analysis, Structural Equation Modeling

Abbreviations

(AGFI) Adjusted Goodness-of-Fit Index, (CFI) Comparative Fit Index, (GFI) Goodness of Fit Index, (IFI) Incremental Fit Index, (LISREL) Linear Structural Relations, (KMO) Kaiser Meyer Olkin, (MI) Modification Index, (MIMIC) Multiple Indicators and Multiple Causes, (NFI) Normed Fit Index, (NNFI) Non-Normed Fit Index, (RFI) Relative Fit Index, (RMSEA) Root Mean Square Error of Approximation, (PRELIS) Preprocessor for LISREL, (PCA) Principal Component Analysis.

1 Introduction

The SDQ, devised by R. Goodman (1997), is a screening tool aimed at identifying the prevalence of social, emotional and behaviour difficulties (SEBD) among children. The SDQ comprises five subscales that measure emotional, conduct, hyperactivity and peer problems, together with prosocial behaviour. Each subscale has five items, all measured on a 3-point scale ranging from 0 to 2, where 0 corresponds to 'Not True', 1 to 'Somewhat True' and 2 corresponds to 'Certainly True'. Five of the items are reverse-coded since they are negatively worded. The score of each subscale ranges from 0 to 10; while the total difficulty score, which excludes the prosocial subscale ranges from 0 to 40. There are three versions of the SDQ; one is administered by the teacher, one by the parent and the other is self-administered by the student. These three SDQ versions have been translated in several languages, including Maltese. Cefai, Cooper and Camilleri (2008) validated the Maltese SDQ version through a process of forward and backward translations. The Maltese and English versions of the SDQ were administered to a number of teachers, allowing a two-week period between the administrations of the two versions.

**Correspondence to*: Liberato Camilleri (liberato.camilleri@um.edu.mt)

The reliability of the Maltese SDQ version was measured item by item, where correlations ranged from 0.82 to 0.98.

Despite the strong clinical use of the SDQ worldwide, several studies yielded mixed results in its structural validity. A number of researchers (Becker, Woerner, Hasselhorn & Banaschewski, 2004; Hawes & Dadds, 2004; R. Goodman, 2001; Smedje, Broman, Hetta & von Knorring, 1999) supported the R. Goodman (1997) five-factor model, by using principal component analysis on a forced five-factor structure solution. They found that each item factor loading weighed heavily on one subscale and cross-loading across subscales was minimal. Other studies supporting the five-factor structure include Ruchkin, Koposov and Schwab-Stone (2007) using the Belgian parent and teacher informant version of the SDQ, Van Leeuwen and Tyukin (2006) using the Russian self-report version of the SDQ and Woerner, Becker and Rothenberger (2004) using the German parent informant version of the SDQ. On the other hand, contrasting results were observed when the number of factors was unspecified. A study carried out by Koskelainen, Sourander and Vauras (2001) reported a three-factor solution, using the self-report version of the SDQ among 1458 Finnish adolescents aged 13–17 years. This factor solution combined the conduct with the hyperactivity subscale and the emotional with the peer subscale to form the externalisation and internalisation dimensions, while the prosocial dimension was retained. This three-factor model structure was also supported by Dickey and Blumberg (2004), who administered the parent SDQ version to a sample of 9574 parents of American children and adolescents aged 4–17 years. The three-step analytic procedure included PCA, EFA and CFA. The authors acknowledge that their failure to replicate the predicted five-factor solution observed in European samples might be attributed to the fact that several items were modified to be more understandable to American parents and indicative of behaviours of their children. Mellor and Stokes (2007) remarked that the five-factor CFA structure did not lead to an acceptable model fit when using the Norwegian self-report version of the SDQ and the Australian parent and teacher informant version of the SDQ. A. Goodman and Goodman (2010) highlighted that 'there is theoretical and empirical support' for an alternative three-factor structure.

2 Theory

The main objective of this study is to analyse the factor structure underlying the rating scores provided to the 25 SDQ items by means of Exploratory Factor Analysis (EFA), Confirmatory Factor Analysis (CFA) and Structural Equation Modelling (SEM). EFA accounts for patterns of correlations existing among the observable variables in terms of smaller number of latent variables. In other words, EFA identifies the latent traits that influence the rating scores provided to the items. Once the factor structure is determined by EFA, the CFA model is fitted to verify the pattern of the factor loadings, the number of underlying dimensions (factors) and any covariances between the factors. Once CFA confirms the latent structure, the SEM model is fitted to assess the relationships between the latent variables. SEM is a statistical technique that assesses unobservable latent traits. It includes a measurement model that defines latent constructs, using several observable variables and a structural model that assigns relationships between the latent variables. Kaplan (2000) describes SEM as 'a class of methodologies that represent the hypotheses involving the means, variances and covariances of the observed data in terms of a smaller number of structural parameters which are defined by means of a hypothesized underlying model'. The links between constructs of a structural equation model can be estimated using the statistical software AMOS or LISREL.

2.1 The Factor Model

Let $\mathbf{X}$ be a set of p observable random variables with mean vector $\boldsymbol{\mu}$ and variance-covariance matrix $\boldsymbol{\Sigma}$. Suppose that $\boldsymbol{\Lambda}$ is a $(p \times q)$ matrix of factor loadings, $\boldsymbol{\eta}$ is a q-random vector of latent factors and $\boldsymbol{\varepsilon}$ is a p-random vector of error terms. If $q < p$, the q-factor model holds for $\mathbf{X}$ and is given by:

$$\mathbf{X} - \boldsymbol{\mu} = \boldsymbol{\Lambda}\boldsymbol{\eta} + \boldsymbol{\varepsilon}. \tag{1}$$

The following assumptions are imposed on $\boldsymbol{\eta}$ and $\boldsymbol{\varepsilon}$

$$\begin{aligned} &E(\boldsymbol{\eta}) = E(\boldsymbol{\varepsilon}) = \mathbf{0} \\ &\mathrm{Var}(\boldsymbol{\eta}) = \mathbf{I} \text{ and } \mathrm{Var}(\boldsymbol{\varepsilon}) = \boldsymbol{\Psi} \end{aligned} \tag{2}$$

where $\boldsymbol{\Psi}$ is a diagonal matrix with diagonal elements ψ_{ii} and $\mathbf{0}$ is the null vector/matrix. Under an EFA model, $\boldsymbol{\Sigma}$ is related to $\boldsymbol{\Lambda}$ and $\boldsymbol{\Psi}$ by:

$$\boldsymbol{\Sigma} = \boldsymbol{\Lambda}\boldsymbol{\Lambda}' + \boldsymbol{\Psi}. \tag{3}$$

Consequently, the variance of $\mathbf{X}$ can be divided into two parts. One component includes the variance explained by the factors and the other includes the unexplained variance. If σ_{ii} is the variance of random variable X_i and $\lambda_{ij} = [\boldsymbol{\Lambda}]_{ij}$, then:

$$\sigma_{ii} = \sum_{j=1}^{q} {\lambda_{ij}}^2 + \psi_{ii}. \tag{4}$$

where $\sum_{i=1}^{q} {\lambda_{ij}}^2$ known as the communality, give the variance of X_i which is shared with the other variables

through the common factors, while the specific variance ψ_{ii} explains the variability of X_i which is not shared with the other variables. Moreover, ${\lambda_{ij}}^2 = \mathrm{Cov}(X_i, \eta_j)$ which shows the extent to which the i^{th} observable random variable X_i depends on η_j.

2.2 The Structural Equation Model

The general Structural Equation Model comprises two models: the measurement model and the structural model. The former model is obtained using CFA; while the latter model is obtained through SEM. CFA tests how well the observable variables represent the smaller number of latent constructs. CFA confirms both the number of the underlying dimensions of the factors and the pattern of the factor loadings obtained at the exploratory stage. The main steps involved in conducting CFA are *model specification*, *model identification*, *model estimation*, *model evaluation* and if needed, *model respecification*. The CFA model is defined as:

$$\mathbf{X} = \boldsymbol{\xi}\boldsymbol{\Lambda}_{\mathbf{X}} + \boldsymbol{\delta} \tag{5}$$

where $\mathbf{X}$ represents the vector of observed variables, $\boldsymbol{\xi}$ is the vector of latent variables, $\boldsymbol{\Lambda}_{\mathbf{X}}$ is a matrix of coefficients which describe the influence of the latent variables on the observed variables, and $\boldsymbol{\delta}$ is the vector of measurement errors. By convention, all variables in $\mathbf{X}$ and $\boldsymbol{\xi}$ of model (5) are assumed to be written as deviations from their means. Also, $\boldsymbol{\delta}$ is deemed to be uncorrelated with and it is also assumed that $E(\boldsymbol{\delta}) = \mathbf{0}$ and $E(\boldsymbol{\xi}\boldsymbol{\delta}') = \mathbf{0}$.

Each column of $\boldsymbol{\Lambda}_{\mathbf{X}}$ provides the factor loadings of a particular latent variable and the element $\lambda_{ij} = [\boldsymbol{\Lambda}_{\mathbf{X}}]_{ij}$ specifies the load of the i^{th} variable on the j^{th} factor. Under a CFA model, the variance-covariance matrix of $\mathbf{X}$ takes the form:

$$\boldsymbol{\Sigma} = \boldsymbol{\Lambda}_{\mathbf{X}}\boldsymbol{\Phi}{\boldsymbol{\Lambda}_{\mathbf{X}}}' + \boldsymbol{\Theta}_{\boldsymbol{\delta}} \tag{6}$$

where $\boldsymbol{\Phi}$ is the variance-covariance matrix of the latent factors $\boldsymbol{\xi}$ and $\boldsymbol{\Theta}_{\boldsymbol{\delta}} = E(\boldsymbol{\delta}\boldsymbol{\delta}')$ is the variance-covariance matrix of the measurement errors $\boldsymbol{\delta}$. If the parameters are known and the model is correct, the population variance-covariance matrix will be reproduced exactly.

The model presented in (5) takes into account only one set of latent variables $\boldsymbol{\xi}$. In practice, we may have both exogenous and endogenous latent variables. An exogenous variable is an alternative way of referring to an exploratory variable. A variable is exogenous if its causes lie outside the model, that is, it is not caused by some other variable in the model. A variable is endogenous if it is determined by variables within the model; however it is only partially explained by the model. If both types of latent variables are taken into account then model (5) has to be redefined to include the endogenous variables $\boldsymbol{\eta}$. Let $\mathbf{Y}$ represent the p-vector of observed dependent variables. The measurement model for $\mathbf{Y}$ is given by:

$$\mathbf{Y} = \boldsymbol{\Lambda}_{\mathbf{Y}}\boldsymbol{\eta} + \boldsymbol{\varepsilon} \tag{7}$$

where $\boldsymbol{\eta}$ is a an m-vector of endogenous latent variables, $\boldsymbol{\Lambda}_{\mathbf{Y}}$ is a $(p \times m)$ matrix of model coefficients relating $\boldsymbol{\eta}$ and $\mathbf{Y}$ and $\boldsymbol{\varepsilon}$ is a p-vector of errors terms for $\mathbf{Y}$. By convention, all variables in $\mathbf{Y}$ and $\boldsymbol{\eta}$ of model (6) are assumed to be expressed as deviations from their means. It is also assumed that $\boldsymbol{\varepsilon}$ is uncorrelated with $\boldsymbol{\eta}$ and that $E(\boldsymbol{\varepsilon}) = \mathbf{0}$ and $E(\boldsymbol{\eta}\boldsymbol{\varepsilon}') = \mathbf{0}$. The variance-covariance matrix of $\mathbf{Y}$ takes the same form as (6). The structural equation for the latent variable model is given by:

$$\boldsymbol{\eta} = \mathbf{B}\boldsymbol{\eta} + \boldsymbol{\Gamma}\boldsymbol{\xi} + \boldsymbol{\zeta} \tag{8}$$

where $\boldsymbol{\eta}$ is an m-vector of latent endogenous variables, $\boldsymbol{\xi}$ is a n-vector of latent exogenous variables, $\boldsymbol{\Gamma}$ is an $(m \times n)$ coefficient matrix for the latent exogenous variables, $\mathbf{B}$ is an $(m \times m)$ coefficient matrix for the latent endogenous variables and $\boldsymbol{\zeta}$ is an m-vector of errors (disturbances). There are a number of assumptions underlying the structural model defined in (8). One of the model assumptions is that the matrix $(\mathbf{I} - \mathbf{B})$ exists and is non-singular. Two other assumptions are that the error terms ζ_is are uncorrelated with the exogenous variables in $\boldsymbol{\xi}$ and that $E(\zeta_i) = 0$. Another underlying assumption of the structural model is that ζ_i is homoscedastic and not auto-autocorrelated. The structural model comprises two variance-covariance matrices: $\boldsymbol{\Phi}$ is an $(n \times n)$ variance-covariance matrix of the latent exogenous variables $\boldsymbol{\xi}$ and $\boldsymbol{\Psi}$ is an $(m \times m)$ variance-covariance matrix of the error terms $\boldsymbol{\zeta}$.

3 Methodology

To carry out this study, a random sample of 1326 teachers was selected from 110 schools, of which 66 were primary and 44 were secondary schools, to investigate the social emotional and behaviour difficulties of 5200 students, using the teacher SDQ version. The random sample, which was collected in 2005–2006, comprised around 7% of the whole Maltese student population aged 6–16 years. To guarantee a representative sample, the students were stratified by gender, school-level, school-type and school region. The teachers were asked to assess the children under their supervision on each of the 25 items of the Maltese SDQ teacher version.

The five items related to emotional difficulties assessed anxiety, depression, fear and unhappiness. The five items related to hyperactivity assessed restlessness, inattention, distraction, over-activity and inability to finish work. The five items related to conduct problems assessed ill-temper and behaviour problems such as fighting, cheating, lying and stealing. The five items related to peer problems assessed poor relations with

peers, bullying and loneliness and the five items related to prosocial behaviour assessed good qualities, such as being considerate, helpful, caring and kind to others.

After reverse-coding the five negatively worded items, the data was analysed using the facilities of the statistical software SPSS and LISREL. Cefai et al. (2008, 2009), Cefai and Camilleri (2011) estimate the prevalence of SEBD in Malta and identify the risk factors associated with social, emotional and behaviour difficulties. The studies revealed that according to teachers, 81.7% of the students had no to mild social emotional problems, 8.6% had moderate SEBD and the remaining 9.7% had severe difficulties. Males scored significantly higher than females in conduct and hyperactivity problems; whereas females scored significantly higher than males in emotional difficulties and prosocial behaviour. Moreover, the study shows that children with poor attainment and learning difficulties, who have health problems and receive psychological/educational interventions, are more likely to show SEBD problems. To accommodate the hierarchical structure of the data, where students are nested in classroom, which in turn are nested in schools, Camilleri, Cefai and Cooper (2011) use multilevel modelling to identify the significant risk factors of SEBD.

4 Data Analysis and Results

The aim of this paper is to confirm the 3-factor models using the teacher SDQ data collected in 2005–2006. Exploratory Factor Analysis, Confirmatory Factor Analysis and Structural Equation Modelling are used to identify the best model fit.

4.1 Internal Consistency

Cronbach Alpha (Cronbach, 1951) was utilised to assess the internal consistency of the items within every subscale. The items in the Conduct, Hyperactivity, Emotional and Prosocial subscales have satisfactory internal consistency and their Cronbach Alpha exceeded the 0.7 threshold values. The Peer subscale had a weak internal consistency, since its Cronbach Alpha just exceeded the 0.5 threshold. The item *Child gets on better with adults than children of same age* was weakly related to other items in this subscale.

4.2 Exploratory Factor Analysis

Exploratory Factor Analysis was used to assess the factorial validity of the whole SEBD data and to identify the number of latent dimensions underlying this dataset. Firstly, the Kaiser Meyer Olkin (KMO) measure of sampling adequacy was computed and Bartlett's test of sphericity was carried out to establish the presence of a latent structure. The KMO value, which gives an indication of the relative compactness of the correlations, was equal to 0.898, which exceeds the 0.5 threshold value. The Bartlett's test of sphericity, which tests whether the correlation matrix is significantly different from the identity matrix, yielded a p-value less than the 0.05 level of significance. Both results indicate a latent structure within the SEBD data and that EFA is essential to reveal this latent factor structure.

EFA was then carried out through the facilities of SPSS, using maximum likelihood estimation and Varimax rotation. This orthogonal rotation of the factor axes normally makes it easier to identify each observable variable with a single factor. The Kaiser's eigenvalue greater than 1 rule (Kaiser, 1960) and the scree plot identified three underlying factors. Since the rating scores are ordinal categorical responses, polychoric correlations are more appropriate to assess the relationships between the observable items than Pearson correlations. Consequently, the PRELIS interface available in the statistical software LISREL was used to compute the polychoric correlations of this dataset. The polychoric correlations ranged from 0.378 to 0.859 for the Emotional subscale items, 0.340 to 0.646 for the Hyperactivity subscale items, 0.320 to 0.728 for the Conduct subscale items, 0.080 to 0.534 for the Peer subscale items and 0.576 to 0.681 for the Prosocial subscale items. Undoubtedly, the Peer construct is the weakest structure; however, the Chi square test shows that most of the polychoric correlations are significantly different from 0. Moreover, the RMSEA values which assess the normality assumption of the underlying bivariate distributions are small and do not exceed 0.1, which implies no complications due to non-normality.

Violation of the bivariate normality assumption between two variables can cause complications in estimation if the RMSEA value exceeds 0.1 (Jöreskog & Sörbom, 2001). Analysis on the SEBD data shows that only two RMSEA values exceed 0.1, which include *Kind to kids* and *Caring* (0.118), *Kind to kids* and *Shares with others* (0.103). Thus CFA and SEM procedures were based on polychoric correlations.

The next step consisted in conducting EFA using the MINRES facility implemented in software LISREL (version 8.80), by fitting a three-factor model to the dataset using *varimax rotation*. Table 1 displays the factor loadings of this three-factor model. Stevens (2002) suggested a threshold value of 0.4 for these factor loadings when the sample size exceeds 150 observations and the number of variables exceeds 10. Factor 1, which represents the Externalisation dimension, comprises nine of the items in the Hyperactivity and Conduct subscales, including *Tempers*, *Obedient*, *Fights*, *Lies*, *Restless*, *Distractible*, *Fidgety*, *Reflective* and *Persistent*. The item *Steals from home, school or elsewhere*, which was included in the R. Goodman (1997) Conduct subscale, does not feature in the above Externalisation dimen-

Table 1: The factor loadings of three factors obtained through Varimax Rotation

Variable Description	Externalisation Factor	Prosocial Factor	Internalisation Factor
Tempers	**0.54**	0.20	0.08
Obedient	**0.56**	0.27	0.10
Fights	**0.62**	0.21	-0.01
Lies	**0.55**	0.27	0.09
Steals	0.22	0.12	0.11
Somatic	0.21	0.03	0.38
Worries	−0.04	−0.05	**0.62**
Unhappy	0.16	0.08	**0.60**
Clingy	0.12	0.06	**0.60**
Fears	-0.03	0.04	**0.67**
Restless	**0.77**	−0.04	−0.07
Fidgety	**0.80**	0.01	0.01
Distractible	**0.60**	0.20	0.27
Reflective	**0.54**	0.35	0.15
Persistent	**0.48**	0.31	0.27
Solitary	−0.11	0.25	**0.45**
Good Friend	0.05	0.25	0.22
Popular	−0.03	0.39	0.28
Bullied	0.09	0.12	**0.42**
Best with adults	0.12	−0.02	0.20
Considerate	−0.34	**−0.67**	−0.01
Shares	−0.20	**−0.64**	−0.06
Caring	−0.18	**−0.69**	−0.03
Kind to kids	−0.24	**−0.61**	−0.02
Helps out	−0.21	**−0.66**	−0.05

sion. This may be partly attributed to the fact that teachers are not aware of what children do at home. In fact, the vast majority of the teachers disagreed with this item, irrespective of the child's behaviour at school. Factor 2, which comprises all the items in Prosocial subscale, includes *Considerate*, *Shares*, *Caring*, *Kind to kids* and *Helps out*. Undoubtedly, the Prosocial construct is the stronger structure. Factor 3, which represents the Internalisation dimension, comprises six of the items in the Emotion and Peer subscales, including *Worries*, *Unhappy*, *Clingy*, *Fears*, *Solitary* and *Bullied*. The items *Has at least one good friend*, *Liked by other people of same age*, *Gets a lot of headaches stomach aches* and *Gets on better with adults than children of same age* which were included in the R. Goodman (1997) Peer and Emotion subscales, do not feature in the above Internalisation dimension. The items were the least related to other items in their respective subscales.

4.3 Confirmatory Factor Analysis (CFA)

A three-factor CFA model was then fitted to the whole SEBD sample, using the Weighted least squares (WLS) estimation technique. This is the appropriate estimation technique when analysing ordinal categorical responses (rating scores). The fitted model defines the relationships between the Externalisation, Prosocial and Internalisation dimension, whilst relaxing some of the assumptions posed in EFA. Various models were tested. Once a model was specified, the t-rule was used to assess whether the model is identified. Since the t-value for the model fit was 43, which is less than the $0.5q(q+1) = 210$ criterion, then the three-factor CFA model has model identification.

The model parameters were estimated, followed by a quality check of the model fit. The chi-square value, corrected for non-normality, did not satisfy its threshold criterion. With 165 degrees of freedom, the chi-square value (3362.7) yielded a very small p-value (less than 0.0001), which implies that the specified CFA model is not supported by the sample variance-covariance matrix. It should be noted, however, that the chi-square statistic inflates considerably with an increase in the sample size and is not useful for large data sets (Schumacker & Lomax, 2004). Moreover, most of the fit indices did not exceed their threshold values. According to Steiger (2007), Hu and Bentler (1999), Mac Callum, Browne and Sugawara (1996), Klein (2005), a good fit is achieved if CFI ≥ 0.95, TLI ≥ 0.90, RMSEA ≤ 0.06, GFI ≥ 0.90, NFI ≥ 0.95 and SRMR ≤ 0.07; an acceptable fit is achieved if CFI ranges between 0.90 and 0.95 and RMSEA ranges between 0.06 and 0.08.

To improve the model fit, a number of paths were added to the CFA model. The modification of indices (MI) facility, available in LISREL, displays the change in the chi square value when the model fit is modified. The first modification was the addition of an error covariance in the path diagram between the observed variables *Fidgeting* and *Restless*. These two terms have a similar meaning and are often interchanged inadvertently in speech. The corresponding MI value (345.6) is large and may indicate that these two observed variables may produce a sub-dimension within the Externalisation dimension. The second modification was the inclusion of an error covariance in the path diagram between the observed variables *Bullied* and *Solitary*. The corresponding MI value (120.3) is large, indicating a strong perceived link between bullied and solitary students. The three-factor CFA model was re-fitted using these two modifications. The t-value for the best model fit was 45, which is less than the $0.5q(q+1) = 210$ criterion, implying that this fitted three-factor CFA has model identification. The resulting parameter estimates of lambda-x, phi-paths and theta-deltas were all found to be significant since the corresponding z-scores exceed 1.96 for all observed variables.

Figure 1 displays the path diagram and corresponding WLS estimates of the three-factor CFA model. The path diagram shows the relationships between the three dimensions (Externalisation Internalisation and Prosocial factors) and their relationships with the twenty observed items.

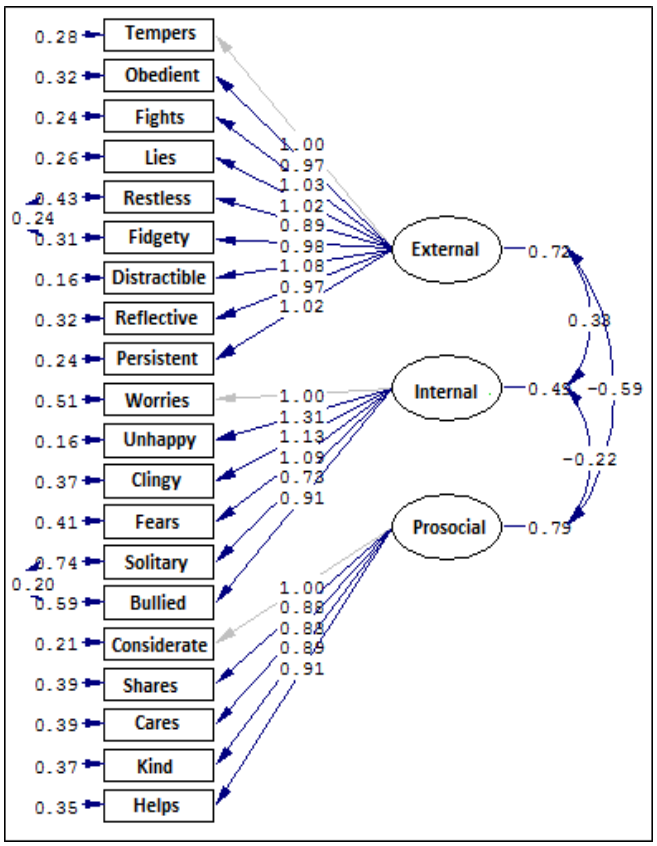

Figure 1: Path diagram of the three-factor CFA Model 1.

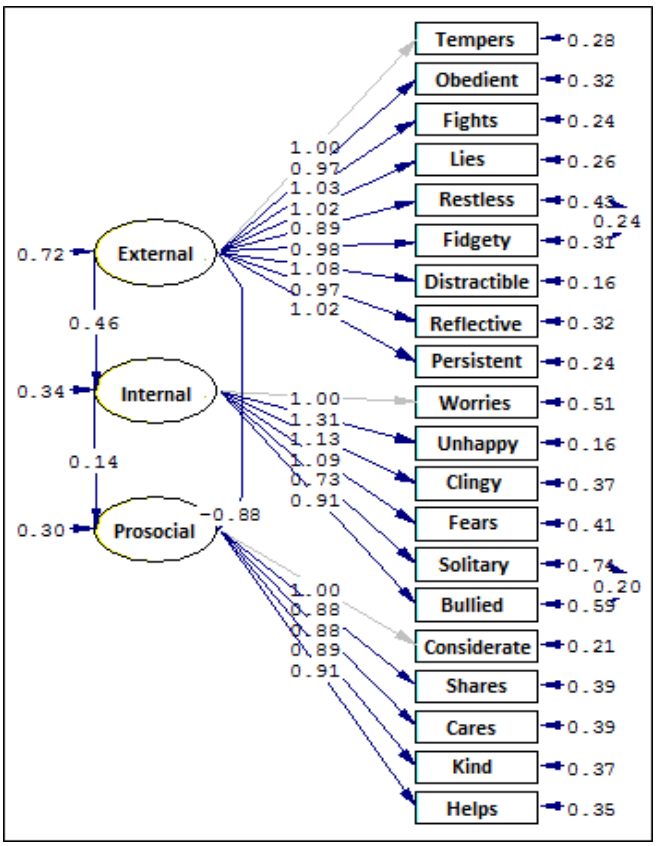

Figure 2: Path diagram of the three-factor SEM Model 2.

The Externalisation factor respectively explained 84%, 77%, 76%, 74% and 71% of the variances of the items *Distractible*, *Fight*, *Persistent*, *Lies* and *Tempers*. The Internalisation factor explains 91% of the variance of the item *Unhappy* and the Prosocial factor explains 79% of the variance of the item *Considerate*. The majority of the standardized factor loadings exceed 0.7, indicating that the latent factors strongly affect 18 of the observed variables and moderately affect the remaining 2 items: *Solitary* and *Bullied*. Furthermore, the CFI (0.93), GFI (0.98), AGFI (0.97), NFI (0.92), NNFI (0.92), IFI (0.93) and RFI (0.91) all exceed their threshold values indicating a well-fitted model. Moreover, the Hoelter's Critical N (393.5) exceeds the 200 cut-point and the RMSEA value (0.06) is less than the 0.07 threshold value suggested by Steiger (2007). All these fit indices satisfy their threshold criteria, which indicate that this three-factor CFA model (Model 1) fits the data well.

4.4 Structural Equation Modeling (SEM)

A three-factor structural equation model was also fitted on the dataset using LISREL to investigate the relationships between the latent variables. Essentially, this involves regressing latent variables on one another.

Figure 2 displays the path diagram of this three-factor SEM model, which displays the relationships between the three factors (Externalisation, Internalisation and Prosocial) and their relationships with the 20 observed items. Once the model is specified, the t-rule was used to check that the three-factor SEM has model identification. The model parameters were estimated using a weighted least squares estimation procedure. The corresponding factor loadings, phi-paths and theta-deltas estimates are all significant since their standard errors are less than half the value of the parameter estimates. Although in the final SEM the direct effect of the Externalisation factor on the Prosocial factor is strongly negative, there is an indirect positive effect through the Internalisation factor. Consequently, the total effect of the Externalisation factor on the Prosocial factor is equal to $-0.816\,(-0.88 + 0.46 \times 0.14)$. This implies that students who score high on externalising behaviour problems tend to score low on prosocial behaviour.

Figure 3 displays the completely standardized solution of the three-factor SEM model. Since none of these standardized estimates exceeds 1 in absolute value, then the solution is deemed to be acceptable. The CFI (0.92), GFI (0.98), AGFI (0.97), NFI (0.92), NNFI (0.91), IFI (0.92) and RFI (0.91) all exceed their threshold values

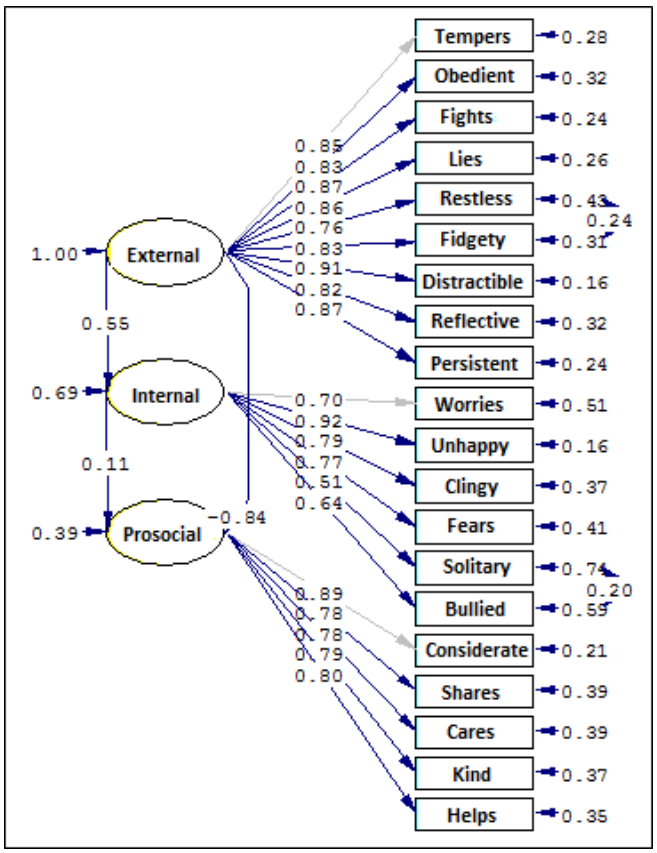

Figure 3: Standardised solution of the three-factor SEM Model.

by a small margin indicating a plausible fit. Furthermore, the Hoelter's Critical N (325.96) exceeds the 200 cut-point and the RMSEA value was (0.061) which is less than the 0.07 threshold value suggested by Steiger (2007). Although the SRMR value (0.18) exceeds the 0.1 criterion, it is still fairly close to 0. The chi-square value for the three-factor SEM model corrected for non-normality does not satisfy its threshold criterion, since the p-value is less than 0.05. However, this result will be ignored in light of the favourable results obtained from the various fit indices and the fact that large samples tend to yield large chi-square values. These results indicate that this three-factor SEM model fits the data well and achieves an acceptable level of construct validity.

The percentage variation of the Internalisation factor explained by the Externalisation factor is 31%, and the percentage variation of the Prosocial factor explained by the Externalisation and the Internalisation factors is 61%. The Externalisation factor respectively explains 84%, 76%, 76%, 74% and 72% of the variances of the items *Distractible*, *Fight*, *Persistent*, *Lies* and *Tempers*. The Internalisation factor explains 84% of the variance of the item *Unhappy* and the Prosocial factor explains 79% of the variance of the item *Considerate*. Moreover, Figure 3 shows that the Externalisation factor has a strong negative influence on the Prosocial factor (−0.84), which implies that children with externalising behaviour problems are less likely to display prosocial behaviour. Conversely, the Externalisation factor has a positive influence on the Internalisation factor (0.55), which implies that children with externalising behaviour problems are more likely to exhibit internalising behaviour problems. The Internalisation factor has a weak positive influence on the Prosocial factor (0.11), which may indicate that children with internalising behaviour problems tend to be more prosocial than children with externalising behaviour problems.

5 Conclusion

The study supports the three-factor structure model for the SDQ. Cronbach Alpha indicates good internal consistency between the items describing the Emotion, Conduct, Hyperactivity and Prosocial subscales; however, items of the Peer subscale have weak internal consistency. The KMO value and Bartlett's test support the use of factor analysis to identify latent structures within the data. The Kaiser's eigenvalue greater than 1 rule suggested a three-factor model. Moreover, a scree plot displaying the eigenvalues of all components plotted in descending order showed a scree elbow at the fourth component, complementing a three-factor rather than a five-factor model. The three latent factors, which were identified using EFA, load heavily on the items of the Externalisation, Internalisation and Prosocial dimensions. CFA confirms that the three-factor model provides a better fit to the data than the five-factor model proposed by R. Goodman (1997). The Externalisation dimension combines all the items of the Conduct and Hyperactivity subscales, excluding the item '*steals from home, school or elsewhere*'. The Internalisation dimension combines all the items of the Peer and Emotion subscales, excluding the items '*disliked by children of the same age*', '*gets on better with adults than with children of the same age*', '*Gets a lot of headaches stomach aches*' and '*has no good friends*'. On the other hand, the items of the Prosocial dimension were all retained.

SEM investigates the relationships between the three dimensions and identifies the Externalisation dimension as the dominant factor. SEM also reveals that the Externalisation dimension has a strong negative influence on the Prosocial dimension and a strong positive influence on the Internalisation dimension. The influence of the Internalisation dimension on the Prosocial dimension is weakly positive. These results clearly suggest that by targeting student externalisation behaviour problems, teachers and educators could be more effective in reducing student internalising behaviour problems and enhance prosocial attitudes in both primary and secondary

schools.

CFA and SEM models ignore the nested hierarchical structure of the data where students are nested in classrooms, which are nested in schools. A recommendation for future research is to fit a multilevel structural equation model that accommodates both the hierarchical nested structure of the data, but also caters for the latent factor structure in the data. Another recommendation is to include student, classroom, school and home predictors in the model. Chih-Chien (2005) showed how MIMIC (*Multiple Indicators and Multiple Causes*) models can be used to serve this purpose because they accommodate both latent variables and explanatory variables.

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Review Article

Pathobiology of Saphenous Vein Grafts

Joseph Galea*[1]
[1]*Department of Surgery, University of Malta, Msida, MSD 2080, Malta*

Abstract. The long saphenous vein graft is the commonest conduit used for coronary artery bypass surgery. The short and long term success of the procedure depends on the patency of these bypass grafts. Vein graft disease can be divided into early (in the first 30 days), intermediate (1 month to 1 year) and late (over 1 year). Early graft failure is usually caused by graft thrombosis and may be related to the surgical procedure, intermediate graft disease results from intimal hyperplasia while late graft pathology is a consequence of atherosclerosis. The etiology and pathological processes leading to these damaging effects on saphenous vein grafts are tackled in this review. The loss of endothelial integrity, the phenotypic changes in vascular smooth muscle cells and involvement of adventitial cells with collaboration of blood borne factors lead to occlusive pathology of saphenous vein grafts. The accelerated intimal hyperplasia and atherosclerosis are characteristic pathobiological features of these vein grafts. Inflammatory and immunological changes and graft thrombosis are mediated through the secretion and up regulation of growth factors, pro coagulant substances and other proteins arising from the vein wall cells and the blood flowing through them.

Keywords: saphenous vein grafts, vein graft failure, CABG surgery, intimal hyperplasia, graft atherosclerosis, graft thrombosis

1 Introduction

The treatment of coronary artery disease includes a surgical procedure whereby diseased sections of coronary arteries are bypassed using autologous blood vessels or grafts. This technique restores the supply of oxygen and nutrients to match the demands of the myocardium by improving coronary blood flow. This procedure is aptly named coronary artery bypass graft (CABG) surgery. Segments of the long saphenous vein are harvested and then joined to the coronary artery beyond the stenotic lesions [coronary artery-saphenous vein graft (SVG) anastomosis] and to the aorta (aorto-SVG anastomosis) both in an end to side manner (Fig. 1). The success of this bypass depends on the size of the coronary artery, the run-off of blood distal to the graft insertion, the rheology of the blood it contains and the biological characteristics of the conduit itself. Apart from the long saphenous vein, the internal thoracic artery, radial arteries and occasionally the gastroepiploic artery, the short saphenous vein and the arm veins are used to provide the conduit. However, the long saphenous vein graft is still the commonest conduit used in coronary artery bypass graft surgery and the bypass operation for peripheral vascular disease (Allen et al., 2005).

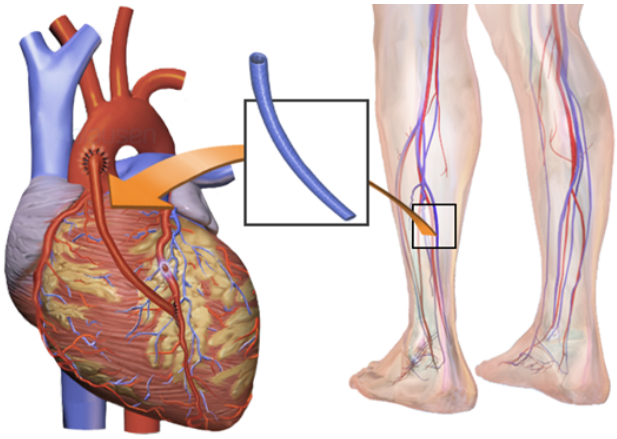

Figure 1: A diagram showing a saphenous vein graft (SVG) anastomosed end to side to the left anterior descending artery (LAD) and to the aorta to bypass a lesion in the proximal segment of the LAD. (Modified from Wikipedia)

Graft failure results from reduction of graft patency and studies have shown that this patency is directly re-

**Correspondence to*: Joseph Galea (joseph.f.galea@um.edu.mt)

lated to the clinical and prognostic outcome of the patients (Halabi et al., 2005; Lopes et al., 2012). Various pathophysiological processes lead to early, intermediate and late SVG disease with consequent stenosis or occlusion of the conduit. Early graft failure, occurring in the first 30 days following operation, happens in 8–18% of vein grafts, usually results from technical problems related to the surgery and is mainly caused by graft thrombosis (Fig. 2) (G. M. Fitzgibbon et al., 1996; Chesebro et al., 1982). Intermediate graft failure takes place from 1 month to 12 months after CABG, has an incidence of 10% and is the result of SVG intimal hyperplasia (Bourassa, Campeau, Lesperance & Grondin, 1982; Sharma, Khuri & Folland, 1982; S. Goldman et al., 2004). Late graft failure (Fig. 3) occurs after 1 year and is the result of ensuing graft atherosclerosis. 25% to 35% of graft would have failed at 5 years (Hess et al., 2014; Campeau et al., 1983) and over 50% at 10 years (Kim, Marhefka, Ruggiero, Adams & Whellan, 2013; C. M. Grondin & Thornton, 1995; Campeau et al., 1983; G. M. Fitzgibbon et al., 1996; S. Goldman et al., 2004).

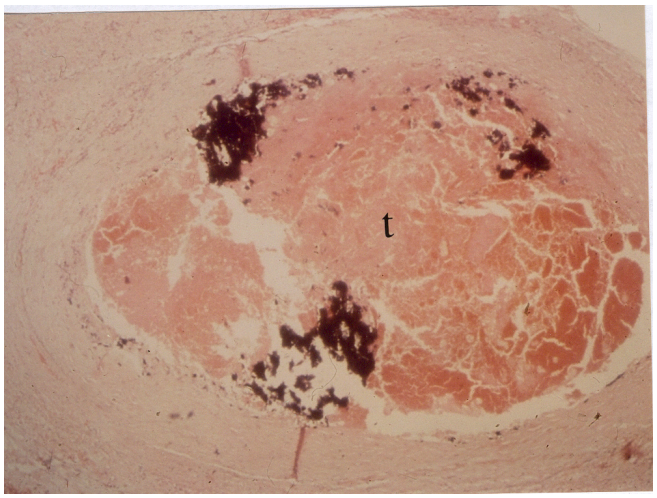

Figure 2: Early graft failure in saphenous vein graft occluded by thrombus (t) in its lumen. (H&E; X12.5 magnification).

2 Early Changes in Saphenous Vein Grafts

Changes in the saphenous vein begin at the time of harvesting, surgical preparation of the vessel and its transplantation into the arterial system. Studies on vein grafts recovered early following bypass graft surgery show that polymorphonuclear (PMN) infiltration into the intima, media and adventitia occurs in the first hours after surgery. This infiltration is an early phenomenon and by 7 to 10 days after bypass graft surgery PMN are virtually absent from the vein graft (Kockx, Cambier, Bortier, De Meyer & Van Cauwelaert, 1992). By 24 hours, most endothelial cells have been denuded

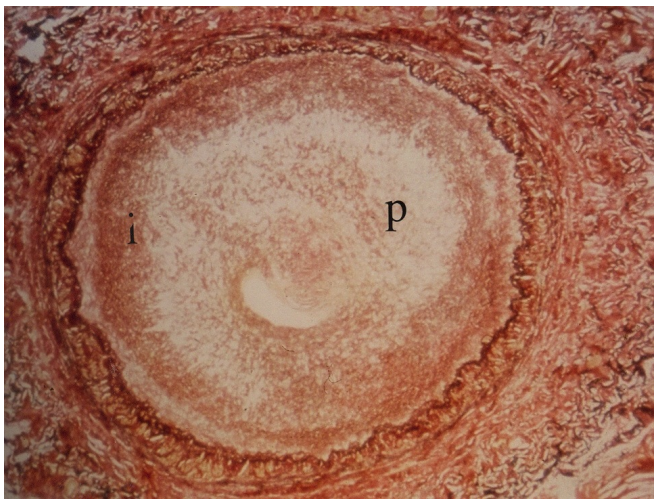

Figure 3: Late graft failure in saphenous vein graft showing intimal hyperplasia (i) and plaque (p) with severe narrowing of the graft lumen (l). (H&E; X 12.5 magnification).

or sloughed (Kockx et al., 1992). Endothelial cell loss may be partly related to injury during surgical preparation of the SV (Gurkan et al., 2014; Angelini, Passani, Breckenridge & Newby, 1987; Angelini, Christie, Bryan & Lewis, 1989). Kockx et al. (1992) suggest that the cause of endothelial denudation is the PMN infiltration however this infiltration could well be the result of chemotactic factors released by damaged endothelium (C. M. Grondin & Thornton, 1995). A recent study by Schlitt et al. (2006) showed that thrombin and mechanical or pharmacological dilatation of human saphenous vein grafts led to increased selectin-mediated PMN adhesion on vascular endothelium with subsequent endothelial dysfunction. Also Chello et al. (2003) showed that substantial distention pressure leads to the expression of endothelial adhesion molecules in human saphenous vein and these in turn cause adhesion of PMNs to the vein wall. High distension pressure leads to endothelial cell separation, causes disruption of the smooth muscle cells in the media (Brindle, 1993) and as soon as there is blood flow the endothelial cells easily wash away leading to possible vasoconstriction. The denuded sections will then be covered with new endothelium 1 to 2 weeks after implantation (Hausmann, Merker & Hetzer, 1996). The denudation of endothelium may induce a cascade mechanism for intimal formation and thickening and this is supported by studies showing that early re-endothelialisation inhibits subsequent intimal thickening in vein grafts (Shiokawa, Rahman, Ishii & Sueishi, 1989; Krijnen et al., 2012). In this acute phase there is also intimal oedema, with deposition of fibrin and platelets onto the endothelium (C. M. Grondin & Thornton, 1995; Chiu & Chien, 2011). Muscle necrosis occurs in the media, which is more marked in its inner circular

smooth muscle layer (Kockx et al., 1992; O'Brien et al., 1998; Cristian et al., 2016). It is suggested that this may occur from medial ischaemia following interruption of the venous vasa vasorum (Brody, Angell & Kosek, 1972) and from direct surgical trauma to the media with induction of an inflammatory response and cell necrosis (Hoch, Stark & Turnispeed, 1995; Mannion et al., 1998; Cristian et al., 2016). Programmed cell death (apoptosis) has also been noted after arterial injury and vein grafting (Kockx et al., 1994; Pearlman, Maillard, Krasinski & Walsh, 1997; O'Brien et al., 1998). The remaining smooth muscle cells in the graft media are of the synthetic phenotype (Kockx et al., 1992) in which state they would respond to various mitogens in contrast to the contractile phenotype, which do not (Thyberg & Fredholm, 1987; Brody, Kosek & Angell, 1972). Growth factors released by the damaged endothelium or injured smooth muscle cells promote platelet aggregation, hyperplasia and the migration of the synthetic smooth muscle cells to the intima resulting into intimal thickening (Brody, Kosek & Angell, 1972; Groves, Kinlough-Rathbone, Richardson, Moore & Mustard, 1979; Packham & Mustard, 1986). PMN, monocytes, lymphocytes and platelets attach to the freely accessible collagen or elastic fibres in the basement membrane (Reidy, 1985). Surgical dissection has been shown to activate adventitial fibroblasts (O'Brien et al., 1997), which in turn migrate through the media to take part in neointimal formation in vein grafts (Shi, O'Brien, Ala-Kokko et al., 1997). This active role of fibroblasts in vascular remodelling is also supported by evidence that extracellular matrix production of the injured adventitia occurs at the same time as neointimal formation (Shi, O'Brien, Mannion et al., 1997).

3 Intermediate and late changes in saphenous vein grafts

3.1 Changes occurring four weeks to one year

The endothelium regenerates after 2 weeks to cover a much thicker intima (Fuchs, Mitchener & Hagen, 1978; Hausmann et al., 1996). Fibrointimal hyperplasia describes the more chronic effects of intimal thickening commencing 1 month after grafting (Barboriak, Pintar & Korns, 1974; Unni et al., 1974; Bulkley & Hutchins, 1977; Kalan & Roberts, 1990). This represents regeneration of the intimal layer, which consists of a thick layer of matrix rich in mucopolysaccharides containing fibroblasts, smooth muscle cells and occasional foam cells (Unni et al., 1974). There is loss of smooth muscle cells from the media and some fibrosis rendering the vein graft rigid (Barboriak et al., 1974; O'Brien et al., 1998). The lumen of the graft becomes narrower angiographically (C. Grondin et al., 1974) and by 1 year its length shortens by about 10% during the first year causing radiological 'tenting' of the distal anastomosis (C. M. Grondin & Thornton, 1995).

3.2 Changes after one year

After the first postoperative year and up to the fourth year, histological changes in the vein graft are small and the lumen to wall thickness ratio stabilises although the graft media and intima may become more fibrous with more matrix and fewer nuclei. However during this period an increase in lipid content and number of foam cells in the vein wall become evident (Kalan & Roberts, 1990). It is believed that lipid deposition, foam cell presence and intimal thickening are the precursors or early signs of atherosclerosis (Bulkley & Hutchins, 1977; Kalan & Roberts, 1990; Vlodaver & Edwards, 1973). Although these histological features may appear as early as 3 months postoperatively (Bulkley & Hutchins, 1977) and at 1 year angiographically (G. Fitzgibbon, Leach, Keon, Burton & Kafka, 1986), clinical manifestations of graft disease appear after 3 years following surgery. The changes are indistinguishable histologically from arterial atherosclerosis (Unni et al., 1974; Bulkley & Hutchins, 1977) but in contrast to atherosclerosis in arteries which has got a life history lasting decades, the disease process in saphenous vein coronary grafts is accelerated and unstable leading to sudden pathological events (Killen et al., 1998; Lytle et al., 1992).

4 Aetiology of vein graft disease

Early graft occlusion occurs within 30 days of coronary artery surgery. It has been shown in experimental and clinical studies that early occlusion is mainly thrombotic in nature (Badimon, Ip, Badimon & Fuster, 1990) while late occlusion usually results from intimal hyperplasia with or without thrombus superimposition and atherosclerosis. Thrombosis, intimal hyperplasia and atherosclerosis seem to occur at different times but they are pathophysiologically interlinked in the evolution of vein graft disease.

4.1 Early vein graft disease and occlusion

The saphenous vein can be damaged during harvesting, surgical preparation and implantation onto the coronary arteries and aorta. The initial damage to the vein endothelium and adventitia occurs during harvesting from the leg and this occurs despite using a 'no-touch' technique and very careful dissection (Gottlob, 1977; Roubos, Rosenfeldt, Richards, Conyers & Davis, 1995; Kim et al., 2013). Vein dissection, handling of the vein with surgical instruments and adventitial stripping are all potentially traumatic. Spasm occurs while the vein is being excised leading to endothelial damage (Baumann, Catinella, Cunningham & Spencer, 1981; Raja & Sarang, 2013). Spasm causes endothelial disruption by reducing the luminal surface area upon which the

endothelial layer lies and by causing smooth muscle cell herniation into the endothelial layer or lumen in a pseudopod-like fashion with subsequent protrusion or sloughing of endothelial cells. Surgical distension of the vein with fluid (e.g. heparinised blood or saline) under pressure to check for any leakages from side branches is known to injure both endothelial and medial cell layers as demonstrated in several quantitative morphological and biochemical studies (Lo Gerfo, Quist, Cantelmo & Haudenschild, 1983; Angelini, Breckenridge, Butchard, Armistead & Middleton, 1985; Roubos et al., 1995; Galea et al., 1999; Khaleel et al., 2012). Endothelial damage leads to decreased production of tissue plasminogen activator (tPA) (Nachman & Silverstein, 1993), fibrin accumulation and adherence (Boerboom et al., 1980), platelet aggregation, neutrophil activation and circulating growth modulation (Reidy, 1992; Verrier & Boyle, 1996; Schlitt et al., 2006), which may result in thrombosis and may subsequently contribute to intimal hyperplasia. Exposure of the prothrombotic basement membrane and other subendothelial tissues occurs following endothelial damage with the release of tissue factor (Verrier & Boyle, 1996) and the activation of the extrinsic pathway of coagulation and subsequent graft thrombosis. Medial damage leads to local inflammation with monocyte infiltration. Adherent monocytes that will have entered the vessel wall change into resident macrophages, which secrete smooth muscle cell mitogens and may accumulate lipid deposits thus contributing to vein graft atheroma. Endothelial injury occurs during distension either as a result of direct mechanical trauma or wall tension (Gottlob, 1977; Angelini, Passani et al., 1987; Lo Gerfo, Quist, Crawshaw & Haudenschild, 1981; Stigler et al., 2012; Dong Li et al., 2014). Vein distension induces phosphorylation of p38 mitogen-activated protein kinase (Cornelissen, Armstrong & Holt, 2004). The acute loss of endothelial-independent function that occurs in de-endothelized saphenous veins with direct effect on the VSMC, may be due to the p38 mitogen-activated protein kinase–mediated degradation of the α-actin filament in venous smooth muscle (J. Goldman, Zhong & Liu, 2003). The smooth muscle cells of the vein graft dedifferentiate from their contractile activity with consequent increases in matrix metalloprotease activity and expression of cytoskeleton-associated proteins, enabling migration and proliferation of the smooth muscle cells (J. L. Johnson, van Eys, Angelini & George, 2001). The storage of the vein in a solution of low oncotic pressure (e.g. normal saline, Hartmann's solution, heparinized saline solution) also causes endothelial injury (Zerkowski et al., 1993; Osgood et al., 2014; Wise et al., 2015). The damage to the endothelium reduces the production of prostacyclin (Angelini, Breckenridge, Psaila et al., 1987) and endothelium-derived relaxing factor (Angelini et al., 1989) both of which normally oppose platelet activation (Radomski, Palmer & Moncada, 1987). Angelini and others showed reduction of short-term patency with endothelial damage and promotion of platelet and leukocyte adhesion in a pig arteriovenous bypass graft model (Angelini, Bryan, Williams, Morgan & Newby, 1990). Other causes for early occlusion of vein grafts include kinking, technical error in proximal and distal anastomosis and sudden exposure of vein graft to high-pressure pulsatile arterial system. Technical errors during suturing may result in obliteration, narrowing, artery wall dissection and tearing of the coronary artery leading to thrombosis and early graft occlusion at the site of the distal anastomosis. Early occlusion from thrombosis is more likely following endarterectomy of the coronary artery where the intima and inner portion of the media are shelled out to increase the diameter of the lumen rendering the distal anastomosis possible. This procedure denudes the endothelium and parts of the media leaving behind a highly thrombogenic surface which is known to promote early graft thrombosis (Walley, Byard & Keon W.J., 1991; Poston et al., 2006). Atherosclerotic disease at the anastomotic site and poor run-off of blood in the coronary artery distal to the anastomosis also predispose to early graft occlusion (Rasmussen et al., 1997).

4.1.1 Graft thrombosis

Vein graft thrombosis follows the pathological process of Virchow's triad namely changes in blood flow, alterations in blood rheology and changes in the vessel wall. Decrease of blood flow occurs at kink sites of vein grafts, or narrowed parts of vein from tight ligatures or distorted anastomoses to the coronaries and the aorta. Early thrombotic occlusion of vein grafts occurs most commonly at these sites. Thrombosis also occurs on vein lumen denuded from endothelium during surgical preparation, leaving the blood in contact with subendothelial collagen, binding and initiating platelet activation (Baumgartner, Muggli, Tschopp & Turitto, 1976). Cardiopulmonary bypass and major surgery alter blood rheology, which may predispose to thombogenicity (Lowe, 1987; Hsu, 1997; Galea, Rebuck, Finn, Manchè & Moat, 1998). CABG surgery alters circulating levels of the coagulation factors with a marked perioperative elevation of plasma fibrinogen favouring a prothrombotic response (Moor et al., 1994; Mannucci et al., 1995).

4.2 The endothelium and graft thrombosis

The vascular endothelium is a key participant in several processes such as coagulation, fibrinolysis, membrane permeability regulation, lipid transport, vasomotor tone, inflammation and the sustenance or alteration of vessel wall structure. The endothelium pro-

duces and secretes proteins which regulate these processes; increases or decreases vascular tone, promotes coagulation or fibrinolysis, enhances or inhibits platelet and leucocyte adhesion or causes structural changes in the vessel wall through the release of growth factors and matrix proteins (Lüscher, Tanner, Tschundi & Noll, 1993; Crossman, Carr, Tuddenham, Pearson & McVey, 1990; Harlan, 1985). The intact endothelium is anti-thrombotic and anti-coagulant under basal conditions and this is achieved through various processes to inhibit coagulation and promote fibrinolysis most markedly NO secretion and the expression of eNOS (Lüscher, Landmesser, von Eckardstein & Fogelman, 2014). The endothelial cells contribute several components of the coagulation cascade. The saphenous vein graft endothelium can be affected by local injurious stimuli (shear stress, surgical injury, hypoxia and cytokines) or by systemic inflammatory processes such as endotoxins and cytokines produced by cardiopulmonary bypass and ischaemia-reperfusion injury (Verrier & Boyle, 1996). Firstly, the endothelial cell does not constitutively express tissue factor (Drake, Morrissey & Edgington, 1989; Crossman et al., 1990). Tissue factor is a 47-kDa membrane bound glycoprotein co-factor which in conjunction with factor VIIa causes the activation of the extrinsic pathway of coagulation (Nemerson, 1988) permitting activation of factors IX and X. Most other cells in the subendothelial layers constitutively express tissue factor therefore exposure of these layers following endothelial injury leads to clot formation at the site of endothelial denudation (Edgington, Ruf, Rehemtulla & Mackman, 1991). Also injurious stimuli to the endothelium lead to up-regulation of tissue factor by the endothelial cells initiating coagulation and there is evidence to suggest that the endothelium supports the coagulation process, whether initiated by the extrinsic or the intrinsic pathways. Endothelial cells carry high-affinity receptors for factors IX and X (Heimark & Schwartz, 1983) and in these cells, IXa binding was potentiated in the presence of factors VIII and X indicating the facilitatory role of the endothelium in the coagulation process (Stern, Nawroth, Kisiel, Vehar & Esmon, 1985). The second endothelial-derived anticoagulant mechanism involves thrombomodulin. Thrombomudulin is a membrane bound anti-thrombotic regulatory protein synthesised by endothelial cells and is expressed on the cellular membrane, where it forms a 1:1 complex with thrombin (Dittman & Majerus, 1990; Esmon & Owen, 1981). This complex activates circulating protein C, which in the presence of its co-factor protein S, causes inactivation of factors VIIIa and Va (Esmon, 1987), destroying their pro-coagulant co-factor activity. The harvesting and preparation of the saphenous vein attenuates the activity of thrombomodulin by up to 30%, further increasing the procoagulant effect (Cook et al., 1991). The overall coagulant status of the cell will then depend upon the relative balances of tissue factor and thrombomodulin activity and the endothelium has developed a complex mechanism that allows it to down-regulate thrombomodulin while up-regulating tissue factor expression at the same time in response to surgical injury, cytokines, endotoxin, hypoxia, shear stress and other stimuli (Nawroth, Handley, Esmon & Stern, 1986; Bevilacqua et al., 1986; Conway & Rosenberg, 1988; Moore, Esmon & Esmon, 1989; Conway, Bach, Rosenberg & Konigsberg, 1989; E. M. Boyle, Verrier & Spiess, 1996). Another important anticoagulant process utilises the potential of the endothelium to express heparin on its surface (Marcum, McKenney & Rosenberg, 1984; Colburn & Buonassisi, 1982). Compared to arteries, the vein media and its poorly developed internal elastic lamina expresses less heparan sulphate (Cox, Chiasson & Gotlieb, 1991) leading to a procoagulant situation. The expression of this anticoagulant proteoglycan is mediated by increased release of antithrombin III. Heparin binds to thrombin reducing the cleavage of fibrinogen to fibrin. It has been suggested that the activation of thrombin, which occurs on the platelet membrane also occurs on the surface of endothelial cells (Rodgers & Shuman, 1983). Thrombin was formed when adding factor Xa and prothrombin to endothelial cells and this process was inhibited by anti-factor V antibody. The production of factor V by the endothelium was substantiated by S35 methionine studies (Cerveny, Fass & Mann, 1984) confirming that the abnormal endothelium plays a role in the thrombin formation. If any clot is formed the constitutive endothelial cell expression of tissue plasminogen activator (tPA) bonds with thrombospondin and the complex catalyses the conversion of plasminogen to plasmin promoting local lysis of the clot forms (van Hinsbergh, 1988; Dichek & Quertermous, 1989; Crossman et al., 1990). However, the injured endothelium upregulates plasminogen-activator inhibitor-1 (PAI-1), which also promotes procoagulant effects.

Apoptosis of the endothelial cells may occur in the vein subjected to the surgical technique and implanted in an extraneous part of the circulation. Apoptotic endothelial cells were shown to increase tissue factor procoagulant activity and decrease antigenic thrombomodulin, heparan sulphates and tissue factor pathway inhibitor levels and their activity. Moreover thrombin formation was also increased in the presence of apoptotic endothelial cells. The procoagulant effect in these cells was caused by increased expression of membrane phosphatidylserine and the loss of anticoagulant membrane components (Bombeli, Karsan, Tait & Harlan, 1997). The final endothelial anticoagulant mechanism is the

constant local secretion of soluble vasoactive products such as nitric oxide (Palmer, Ashton & Moncada, 1988; Lüscher et al., 2014), prostacyclin (MacIntyre, Pearson & Gordon, 1978) and adenosine which maintain vasodilation and prevent platelet aggregation and adhesion (Furlong, Henderson, Lewis & Smith, 1987; Radomski et al., 1987). Production of nitric oxide and prostacyclin is lower in veins than in arteries and their production is further reduced by bypass grafting due to endothelial cell loss or metabolic dysfunction (Angelini et al., 1989). Platelet activation stimulates synthesis of thromboxane A2 (Roth, 1986) and release of dense-granule components including serotonin and ATP and α-granule components including platelet-derived growth factor, platelet factor IV, fibrinogen, fibronectin, von Willebrand factor antigen and β-thromboglobulin (Stenberg & Bainton, 1986). These agents together promote vasoconstriction (Lam, Chesebro, Steele, Badimon & Fuster, 1987), further platelet aggregation (Roth 1986) and hence further thrombin and fibrin generation (Shuman & Greenburg, 1986). The low fluid shear stress in grafted saphenous veins as compared to arteries, reduces the shear-dependent release of tPA, NO and prostacyclin (Allaire & Clowes, 1997; Kabirian, Amoabediny, Haghighipour, Salehi-Nik & Zandieh-Doulabi, 2015).

Surgical technical problems that reduce blood flow through the graft may increase the risk of thrombosis. Saphenous veins, especially when denuded of endothelium are very sensitive to circulating vasoconstrictors, including endothelin-1, which is the most potent of endogenous vasoconstrictors (Ganesh et al., 2016). The plasma levels of soluble endothelin-1 increases steeply at the initiation of cardiopulmonary bypass followed by an additional slower rise during its course (te Velthuis et al., 1996). The resulting venoconstriction may attenuate blood flow through the graft and promote stasis. Furthermore thrombin has a vasoconstrictor effect in the saphenous vein (Yang et al., 1997; Gudmundsdóttir et al., 2008).

4.3 Causes of late graft occlusion

Graft occlusion in the first year after implantation is caused mainly by intimal hyperplasia with superimposed thrombosis (Blaas et al., 2016). Experimental studies suggest that ischaemia, higher intraluminal pressure, and oxygen tension are major causes of fibrous hyperplasia (Brody, Kosek & Angell, 1972). Intimal hyperplasia is believed to represent adaptation of the vein to the trauma of transplantation and although early papers had suggested that intimal hyperplasia may be reduced by aspirin (McCann, Hagen & Fuchs, 1980), this was disproved in later research (Fuster & Chesebro, 1985; Yamaguchi et al., 1991; Landymore, MacAuley & Manku, 1990, 1992) although other anti-platelet therapy may reduce intimal hyperplasia (Göncü et al., 2010; Hermann, Weber & Schror, 2002). Smooth cell proliferation continues after re-endothelialisation occurs and although the endothelium appears morphologically intact there is evidence that it is functionally impaired (Angelini et al., 1989; Komori, Okadome & Sugimachi, 1991). After the first year of implantation, vein occlusion is caused principally by graft atherosclerosis. In 70 to 85% of patients presenting with unstable angina (Chen et al., 1996) and myocardial infarction (Douglas, 1994; Chen et al., 1996) following coronary artery bypass graft the culprit lesion is atherosclerotic vein graft stenosis, often with superimposed thrombosis. The atherosclerosis is rapidly progressive (Yahagi et al., 2016). It has been suggested that this progressive form of atherosclerosis occurring in saphenous vein grafts is either initiated or promoted by intimal injury to the graft (Bulkley & Hutchins, 1977; Smith & Geer, 1983) or by conventional cardiac risk factors particularly hyperlipidaemia with lesser effects from smoking and diabetes (Solymoss, Nadeau, Milette & Campeau, 1988; Neitzel, Barboriak, Pintar & Quresh, 1986).

4.3.1 Accelerated Intimal hyperplasia (AIH)

Intimal hyperplasia is the accumulation of smooth muscle cells and extracellular matrix in the intima and is the major disease phenomenon occurring in the vein graft between one month and one year after implantation that eventually reduces the graft lumen and may have superimposed thrombosis (Zubilewicz, Wronski & Bourriez, 2001). Intimal hyperplasia has been described as a chronic structural change in the graft which process leads to formation of thickened fibrocellular layer between the endothelium and the inner elastic lamina (Dilley, McGeachie & Prendergast, 1988). Various studies have shown that many veins exhibit mild intimal or medial fibrosis before grafting (Thiene et al., 1980) and diffuse intimal hyperplasia develops in all vein grafts and is unavoidable (Campeau, Lesperance & Bourassa, 1984; Fuchs et al., 1978). It is believed to be part of the reparative process that takes place in all vessels after injury (Schwartz, DeBlois & O'Brien, 1995). It can be found as a diffuse layer spread evenly throughout the graft or as a focal lesion found anywhere in the graft (Fuchs et al., 1978) however some studies show a thicker intimal layer at the anastomosis (De Weese & Green, 1980; Madras, Ward, Johnson & Singh, 1981). The process of intimal hyperplasia starts within 24 hours of endothelial injury with medial smooth muscle proliferation in response to a number of cytokines and growth factors released from activated endothelial cells, platelets and macrophages (Holt et al., 1992) with the early involvement of the immediate response gene *c-fos* (Galea et al., 1999). Also, NF-κB mediated signalling is central in gene regulation of numerous cytokines and adhesion molecules involved in AIH (Hu et al., 2002; Miyake, Aoki

& Shiraya, 2006). Indeed, proliferation of intima-bound medial VSMC is pivotal to AIH in vein grafts (Dilley et al., 1988; Schwartz et al., 1995) and in the development of atherosclerosis (Ross, 1986; Stary et al., 1992). Intimal hyperplasia occurs quite quickly in vein grafts, angioplasty and transplantation and is termed accelerated intimal hyperplasia.

In the intact media of blood vessels, the major function of the smooth muscle cell is maintenance of tension via contraction-relaxation (Stadler, Campbell & Campbell, 1989) and the cells are arranged predominantly in a circular fashion. In keeping with their function, the cell cytoplasm contains numerous myofilament bundles whereas synthetic organelles such as rough endoplasmic reticulum are few in number and located at the perinuclear region (J. H. Chamley-Campbell, Campbell & Ross, 1981). Phenotypic modulation is an important initial event in the pathophysiology of intimal proliferation. In the majority of cases the VSMCs must first modulate from the contractile state into a synthetic phenotype before they become secretory and responsive to mitogenic stimulation (J. H. Chamley-Campbell et al., 1981) and capable of migration (J. Chamley-Campbell, Campbell & Ross, 1979; Thyberg, Palmberg, Ksiazek & Sjölund, 1983). Intimal VSMCs have also been shown to originate from adventitial fibroblasts, from bone marrow progenitor cells, pericytes and pre-existing intimal cells (Shi et al., 1996). The endothelial cell plays a regulatory role in intimal growth through a number of growth-inhibitory mechanisms therefore endothelial cell loss during surgical manipulation of the vein modifies these effects. Once the endothelium is denuded, platelets adhere to the vessel wall, spread and degranulate releasing mitogens such as platelet-derived growth factor (PDGF) that stimulate the smooth muscle cells to migrate to the intima. Injured endothelial and smooth muscle cells secrete other mitogens such as basic fibroblast growth factor that stimulates proliferation of SMCs in the media (Clowes, 1991). Plaques of modified smooth muscle cells have been found in the intima as early as nine and ten days following grafting (Brody, Angell & Kosek, 1972; Barboriak, Pintar, Van Horn, Batayias & Korns, 1978). Immunocytochemical studies in diseased aortocoronary vein bypass grafts have shown that the majority of cells within the fibrous intimal thickening in veins are smooth muscle cells and macrophages were seldom seen as compared to atherosclerotic arterial disease (Tsukada, Tejima, Amano, Suzuki & Numano, 1988). The modulated smooth muscle cells continue to proliferate further in the neointima. Later these activated SMCs synthesise large quantities of elastin, collagen and proteoglycans and Thyberg, Nilsson, Palmberg and Sjölund (1985) demonstrated that they produce four to five times the amount of connective tissue compared to the contractile phenotype with collagen production being the most florid (Ang, Tachas, Campbell, Bateman & Campbell, 1990). This contributes to deposition of the extracellular matrix leading to progressive increase in intimal fibrosis with reduction in cellularity (Allaire & Clowes, 1997). The activated SMCs temporarily produce PDGF-like substance, which stimulates intimal growth in an autocrine manner (Nilsson, 1987). It has been shown recently that Polo like kinase 1 (PLK1), a serine/threonine kinase that regulates cell cycle progression and mitosis and its expression and activity is elevated in tissues and cells with high mitotic index might play a critical role in VSMC mitosis in hyperplastic intima of vein grafts (Sur, Swier, Radwan & Agrawal, 2016). The secretory phenotypic cellular cytoplasm contains large amounts of free ribosomes, rough endoplasmic reticulum and mitochondria but very few myofilaments (Thyberg et al., 1983). There is a corresponding decrease in myosin, tropomyosin and actin (Kallioniemi, Jaakkola, Nikkari & Nikkari, 1984). Actin changes its isoform from being mainly smooth muscle α-actin in the contractile phenotype to β-actin that is not predominantly a muscle actin (Rubbia & Gabbiani, 1989). This type of synthetic smooth muscle cells have been described in vein graft intimal thickening (Unni et al., 1974; Dilley et al., 1988), in atherosclerotic plaques (Campbell, Black & Campbell, 1989) and foetal blood cells (Nikkari, Rantala, Pystynen & Nikkari, 1988). Histologically these modified smooth muscle cells in the intima are oriented parallel to the long axis of the vessel (Spray & Roberts, 1977; McGeachie, Prendergast & Morris, 1983). The extracellular space of the intima contains some collagen fibres, also aligned with the direction of blood flow; some microfibrils, 10 µm in diameter and rarely elastin (Fuchs et al., 1978; Brody, Angell & Kosek, 1972). Synthetic SMCs show an increased ability to bind and ingest lipoproteins in vitro (Campbell, Reardon, Campbell & Nestel, 1985). Also, vein grafts incorporate more total lipids than arteries and normal veins in hyperlipidaemic patients (Lie, Lawrie & Morris, 1977). The intima of a normal vein is made up of a single layer of endothelial cells (Rhodin, 1968) and the presence of intimal smooth muscle cells is regarded as the first step to intimal hyperplasia. At 2 weeks after autologous grafting of a vein patch to the rat carotid artery, the venous intimal smooth muscles cells existed in various stages of differentiation (Cuevas & Gutierrez Diaz, 1982) and at 3 weeks the cells had differentiated to contain many myofilaments, dense bodies, caveolae intracellulares and gap junctions. This apparent maturation of modified smooth muscle cells to form a muscular intima has also been described by Unni et al. (1974). In contrast to arterial injury, vein grafts AIH occur mostly after re-endothelialisation of the vessel (Dilley,

McGeachie & Tennant, 1992). One explanatory mechanism is the transient ischaemia occurring in the vein from the time of harvesting to reperfusion after implantation in the arterial system. These adverse conditions induce the formation and secretion of superoxide radicals, which promote SMC proliferation and at the same time depress the endothelial production of antiproliferative agents such as prostacyclin, NO and adenosine (Holt et al., 1993; Rao & Berk, 1992). Also loss of the vasa vasorum blood supply in the vein may lead to ischaemia and fibrosis. The phenotypic modulation in SMCs appears to be reversible depending on their proliferative state (J. H. Chamley-Campbell et al., 1981; Stadler et al., 1989). Modulation into the synthetic state can be inhibited in vitro by a feeder layer of confluent endothelial or contractile SMCs and by heparin (J. H. Chamley-Campbell et al., 1981). In vein grafts, SMCs forming intimal hyperplasia modulate back into the contractile phenotype about 3 months after grafting (Dilley et al., 1988). The ability of SMCs to change back into their contractile phenotype seems to depend also on the length of time the proliferative stimulus was applied for (J. H. Chamley-Campbell et al., 1981). Cells that have been in the synthetic phenotype for a considerable period of time cannot change back to the contractile phenotype and will remain permanently responsive to mitogens and hence maintain the stimulus of intimal hyperplasia and atherosclerotic plaque formation. When vein grafts are implanted in the arterial circulation the vein wall is subjected to increased wall stress, which is also implicated in the aetiology of AIH. This has been shown in animal models where increased wall stress was shown to up regulate vein graft intimal receptors for VSMC mitogen basic fibroblast growth factor which is released by injured endothelial and smooth muscle cells (Nguyen et al., 1994). Vein distension also increases vein diameter reducing mean blood velocity and decrease in shear stress. This reduction in shear stress upregulates the production of mitogens such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and endothelin 1 and downregulates growth inhibitors like transforming growth factor-β and NO, thus inducing smooth muscle cell proliferation and intimal hyperplasia (Cox et al., 1991; Allaire & Clowes, 1997; Mitra, Gangahar & Agrawal, 2006). This increase in growth factor activity in both low and high shear stress conditions leads to the supposition that there is an optimum shear value below and above which AIH occurs (Lemson, Tordoir, Daemen & Kitslaar, 2000). Another mechanism that has been shown to be involved in neointimal formation in a porcine model of saphenous vein involves the role of perivascular fibroblasts. These fibroblasts may infiltrate injured media of arterialised SVGs, differentiate to myofibroblasts, acquiring alpha-smooth muscle actin and take part in neointimal formation. The intima of human saphenous vein grafts, retrieved during repeat coronary artery bypass surgery exhibits the profile of cytoskeletal proteins resembling myofibroblasts seen in porcine vein grafts which suggest that this process is mimicked in the clinical situation (Shi, O'Brien, Mannion et al., 1997).

4.3.2 Accelerated Graft atherosclerosis

Atherosclerotic plaque is not detected until one year after surgery and is rarely observed before the second or third year after implantation (Atkinson, Forman & Vaughn, 1985; Solymoss et al., 1988; Kalan & Roberts, 1990). Plaques are observed histologically in 21% of grafts at a mean of 5 years after operation. The pathogenesis of atherosclerosis includes impaired lipid metabolism and an improper immune response leading to chronic inflammation of the artery or vein graft (Weber & Noels, 2011). The fundamental process of atherosclerosis is similar in native coronary arteries and SVGs but it is rapidly progressive in the latter that is similar to the accelerated atherosclerosis occurring in chronic transplant rejection. This rapid disease process is possibly closely related to chronic endothelial injury and dysfunction (Dilley et al., 1992; E. M. Boyle, Lille, Allaire, Clowes & Verrier, 1997). The atherosclerotic lesions occurring in vein grafts differ morphologically from lesions arising in arteries. Vein graft atherosclerosis tends to be diffuse, concentric, and friable with a poorly developed or absent fibrous cap and little evidence of calcification, whereas native coronary disease is proximal, focal eccentric and non friable with a well developed fibrous cap and frequent calcification (Lie et al., 1977; Kalan & Roberts, 1990; Ratliff & Myles, 1989). Histologically, vein graft atherosclerosis has the foam cells as the principal or even the only cells in the lesion and they appear to erode the thickened intima. Consequently, graft atherosclerosis resembles experimentally induced atherosclerosis in animal models rather than fatty streaks and plaque lesions in human arteries. The presence of these foam cells and inflammatory cells such as multinucleated giant cells has led some researchers to propose an immunological basis for vein graft atherosclerosis (Ratliff & Myles, 1989). The evidence for this is firstly because this form of atherosclerosis is very similar to that occurring in coronary arteries in patients with underlying immunological vascular diseases (Ansari, Larson & Bates, 1985; Bulkley & Roberts, 1975), in laboratory animals with experimentally induced immunological vascular injury (Wissler & Vesselinovitch, 1983; Alexander, Clarkson & Fulgham, 1985) and in experimental and human cardiac transplantation (Kottke-Marchant & Ratliff, 1988; D. Johnson, Gao, Scroeder & Billingham, 1988). Secondarily, patients undergoing aortocoronary bypass have been shown to be immunologically stimu-

lated in the postoperative period (Baker, Cohen, Head, DeShong & Graeber, 1986; De Scheerder et al., 1986). Thirdly, atherosclerotic saphenous vein grafts has shown increased deposition of immunoglobulin (Fitzmaurice & Ratliff, 1990; Schepers et al., 2006). Lipid metabolism in the saphenous vein is relatively atherogenic with increased lipid synthesis and uptake (Larson, Hagen & Fuchs, 1974) and decreased lipolysis (Shafi, Palinski & Born, 1987). The presence of high levels of LDL cholesterol and diabetes are independent factors in SVG atherosclerosis (Yanagawa et al., 2014). In vivo intravascular ultrasound studies suggest that the focal compensatory enlargement observed in atherosclerotic native coronary arteries does not occur in diseased saphenous vein grafts (Nishioka et al., 1996). Graft atherosclerosis is otherwise indistinguishable from the native disease but because of the thin fibrous cap on a lipid-rich lesion they are more fragile and prone to rapture and thrombosis. Apoptosis plays an important role in the development of the atherosclerotic plaque mainly through smooth muscle cell and macrophage death leading to plaque instability (S. Bjorkerud & Bjorkerud, 1996; Bennett & Boyle, 1998). Oxidised low density lipoproteins have been shown to induce apoptosis of smooth muscle cells and macrophages through the modulation of Fas and bcl-2 protein expression (Li, Yang & Mehta, 1998). Superimposition of thrombosis on atherosclerotic vessels is a common occurrence and may lead to recurrent angina or myocardial infarction after CABG. In one study over two thirds of resected vein grafts during coronary reoperations for recurrent symptoms showed late thrombosis (Solymoss et al., 1988). Thrombosis and instability of the plaque in vein grafts can occur through two mechanisms. Plaque macrophages may release matrix-degrading enzymes that weaken the fibrous cap (Libby et al., 1996; Finn, Nakano, Narula, Kolodgie & Virmani, 2010). Foam cells in the diseased saphenous vein graft possibly release a factor that induces smooth muscle cell apoptosis in the atherosclerotic intima depleting the plaque of SMCs. This depletion could promote plaque rapture and thrombosis (Kockx et al., 1996; Kockx & Herman, 2000; Stoneman & Bennett, 2004; Libby, 2008). Complement factor C5a has been implicated in plaque rapture in grafts (Wezel et al., 2014).

The understanding of the detailed pathology of the different stages of SVG disease offers different possible opportunities for researchers and clinicians to find ways to counteract this pathological process and improve short and long term patency of these conduits thus reducing mortality and morbidity from such a common disorder. Although arterial conduits such as the left internal mammary has shown better long term patency, this vessel is mainly used on the left anterior descending coronary artery which is usually a bigger vessel than the others with consequent better flow capacity therefore comparison is difficult. The long saphenous vein is still the commonest conduit used in coronary bypass surgery therefore improvement in its patency rate is essential because it is here to stay.

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Review Article

Role of Protein Structure in Drug Discovery

Rosalin Bonetta[1], Jean-Paul Ebejer[1], Brandon Seychell[2], Marita Vella[2], Thérèse Hunter[2] and Gary J. Hunter[*2]
[1] *Centre of Molecular Medicine and Biobanking, University of Malta, Msida, MSD 2080, Malta*
[2] *Department of Physiology and Biochemistry, University of Malta, Msida, MSD 2080, Malta*

Abstract. Many pharmaceuticals currently available were discovered either during the screening of natural of synthetic product libraries or by serendipitous observation. Such a "random" approach entails testing numerous compounds and developing countless high-throughput screening assays. On the other hand, a "rational" approach involves the structure-based route to drug discovery, where the structure of a target protein is determined. Hypothetical ligands may be predicted by molecular modelling, while movement of a molecule may be predicted by Molecular Dynamics Simulations prior to synthetic chemical synthesis of a particular molecule. Here, we will be discussing protein structure-based approaches to drug discovery.

Keywords: Protein Structure, X-ray crystallography, Molecular Dynamics Simulations, Drug design

1 Introduction

Proteins are complex molecules composed of long strings of twenty different types of amino acid. The length of the string and the order of amino acids are vitally important for the protein to function properly in its biological role. This part of the process of protein function, the gene encoding the protein determines these factors. A single mistake (mutation) in the gene may cause the wrong amino acid to be incorporated into the sequence or a nonsense mutation may cause the protein to be truncated. However, protein function is more directly determined by the protein's three dimensional shape, the protein structure, and the availability of non-protein cofactors.

2 Protein Structure - why is it important?

As can be seen in Fig. 1, a complex protein such as xanthine oxidoreductase (XOR) forms a highly convoluted structure, but one which accommodates cofactors and substrates perfectly. Protein structure is typically determined by one of three methods today; X-Ray crystallography, Nuclear Magnetic Resonance spectroscopy (NMR) or cryoelectron microscopy. The former is the oldest and most commonly used technique, while the latter is only just becoming available for the analysis of proteins at atomic resolution. X-Ray crystallography relies on the ability of the protein to form a regular molecular array and crystallise; a completely biologically unnatural condition for any protein. Even so, it is possible and there are now over one hundred thousand entries in the biological structures databank, RCSB (Deshpande et al., 2005). The advantage of NMR over X-Ray crystallography is that it can be performed in solution (no crystals required) but the major problem is size; NMR cannot be used to determine the structure of large proteins. Electron microscopy will soon be capable of providing structural information about protein as good as X-Ray crystallography, and is performed in solution. Today it can yield protein structures to 2.2 Å (X-Rays typically give resolutions as high as 0.6 to 1.3 Å). In the laboratory of Biochemistry and Protein Science, at the University of Malta, we use X-Ray crystallography to determine protein structure, with crystallisation conditions determined in our laboratory applied and subjected to X-Ray diffraction at the University of Leeds, UK in collaboration with Dr Chi Trinh. We have determined the structures of several superoxide dismutase enzymes and mutants (to a minimum of 1.7 Å) and are currently working to solve the structures of others, in-

Correspondence to: Gary J. Hunter (gary.hunter@um.edu.mt)

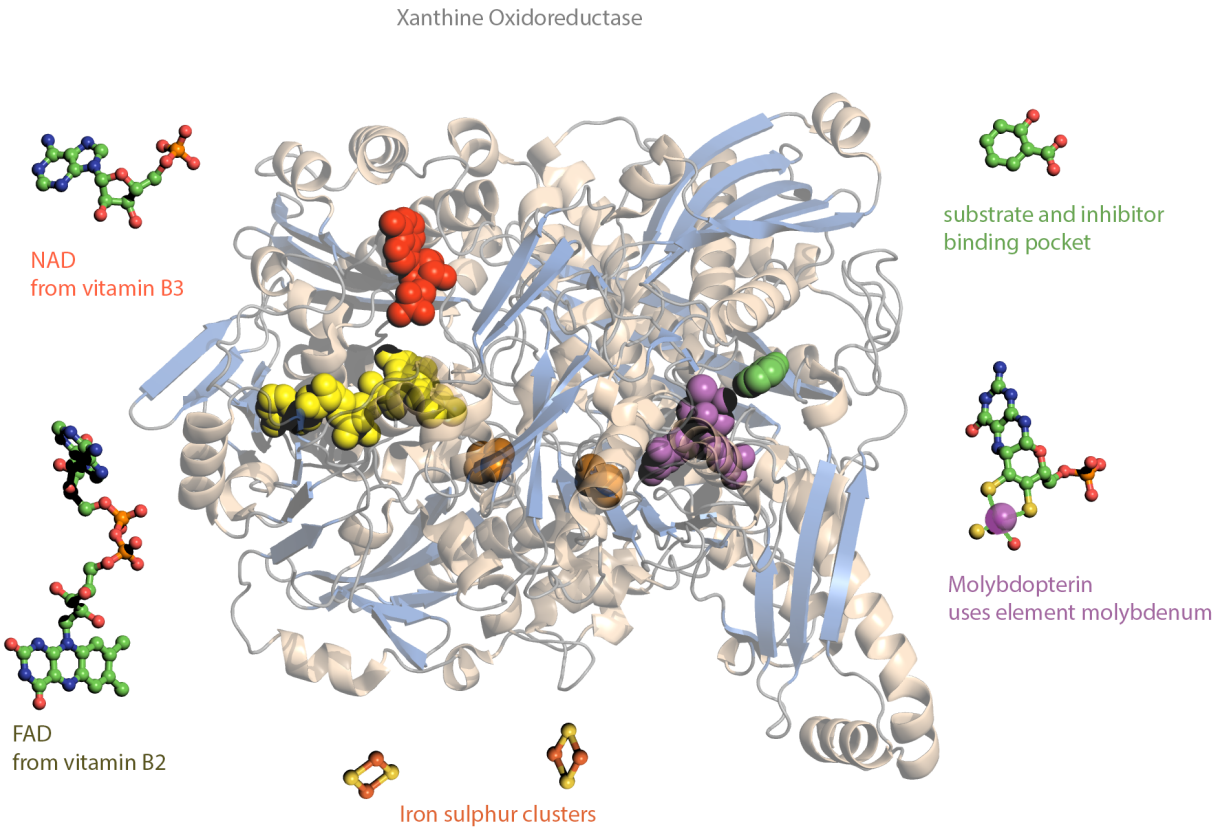

Figure 1: Bovine Xanthine Oxidoreductase. Only one of two identical protein subunits is shown, in cartoon representation with blue beta sheets (arrows) and cream alpha helices (spirals). Cofactors and substrates are shown surrounding the protein in ball-and-stick representation and their corresponding positions of binding within the protein as atomic spheres. NAD is in orange, FAD is in yellow, iron-sulphur clusters are brown, the molybdopterin is purple and xanthine (substrate) in green. Together, the protein forms a scaffold for the cofactors which form an electron transport chain from one side of the protein (substrate) to the other (FAD/NAD). The figure was created using the PyMOL molecular Graphics System (Schrödinger LLC, 2010).

cluding human XOR. Structures such as these help us to understand how the protein functions, and will help to design chemicals to be used pharmaceutically as modifiers of enzyme activity. X-Ray structures usually provide us with a quite static picture of the protein, and it is best combined with other techniques in order to obtain a detailed idea of how the protein functions.

3 Molecular Dynamics Simulations of Biomolecules

Molecular Dynamics Simulations are applied in the investigation of numerous dynamic properties and processes by scientists in a variety of fields that include structural biochemistry, enzymology, biophysics, molecular biology, biotechnology and pharmaceutical chemistry. Molecular Dynamics Simulations allow the researcher to study the thermodynamic and time-dependent (kinetic) properties of biomolecules such as proteins. This provides an understanding of numerous dynamic aspects of biomolecular structure, recognition, and function (Adcock & McCammon, 2006) The techniques involving Molecular Dynamics Simulations involve Langevin's or Newton's equations of motion, as well as a particular molecular bond structure, parametrized force fields, and an initial conformation of atomic positions, together with the velocities that are necessary to generate the atomic dynamics in a molecular system. Molecular Dynamics Simulations have a limited function when used in isolation. The trajectory of Molecular Dynamics (i.e., the progress of a simulated structure correlated to time) usually generates data related only to the level of atomic positions, velocities and single-point energies. Researchers are usually interested in obtaining macroscopic properties. The latter requires the application of statistical mechanics, which combines microscopic simulations together with macroscopic observables. Statistical mechanics provide the mathematical expressions associating the distributions

and motions of atoms to macroscopic observables including free energy, pressure and heat capacity (Callen, 1985; McQuarrie, Salvaterra, De Blas, Routes & Mahler, 1976). Molecular Dynamics Simulation programs include AMBER, CHARMM, NAMD and POLY-MD.

Kinetic rate constants of ligand-receptor interactions are essential in enzymology (Bar-Even et al., 2011) and drug discovery (Copeland, Pompliano & Meek, 2006), as they provide a good indication of drug efficacy (Copeland et al., 2006). Thus, the prediction and optimisation of these parameters is an important challenge in medicinal chemistry (Copeland, 2016). Even though these values may be measured experimentally, an accurate computational prediction would result in a useful alternative in cases where the experiment is either expensive or difficult to perform. Additionally, advances in computational power, have allowed simulations to be carried out in significantly less time. This provides a great potential for methods that require vast amounts of computational power.

Predicting the interaction between an enzyme and its substrate and other ligands via Molecular Dynamics Simulations is essential to fully understand the mechanism of the enzyme. Predicting hydrogen bonding in an enzyme is crucial for analysing the structure and function of this type of biological molecule, especially in terms of enzyme catalysis. Molecular Dynamics Simulations provide information on the molecule that is not observable in the data obtained via X-ray crystallography experiments alone. With this knowledge it is then possible to design new chemicals based upon the binding requirements discovered to inhibit or enhance the biological activity of the protein. In many cases it may be adventitious to modify the structure of an existing, known effector molecule (enhancer or inhibitor) to increase or decrease its activity. With computer aided rational design, a new or modified pharmaceutical may be created with better effectiveness and reduced side effects. Molecular simulations give us the power to suggest or reject such modifications prior to chemical synthesis of the compound. This saves time, effort and money.

4 Protein Structure, Molecular Dynamics, Drug Discovery - tying the knot with computational approaches

Structure-based virtual screening is a computational method employed to find small, bioactive molecules which sterically fit and interact with a protein. A library of small molecules (ligands) is "docked" to the protein's binding site in a typical "lock-and-key" fashion (Meng, Zhang, Mezei & Cui, 2011). Three things are required for this computational approach. Firstly, a protein structure is either determined experimentally (as described earlier) or modelled computationally, typically using homology modelling. In homology modelling, we use one or more known protein structures with close sequence similarity as a template to model our protein of interest. The binding site on the protein needs to be identified. Secondly, a library of small molecules must be prepared and provided to the docking algorithm. This preparation may imply many steps such as sanitisation, setting the appropriate ionization state, removing salts, etc. Thousands to millions of molecules form part of the digital library, only a fraction of which could possibly be tested physically in a laboratory. Thirdly, a docking protocol is required which defines the parameters used in the docking experiment. This includes, but is not limited to, ligand flexibility, protein side-chain flexibility, role of water molecule in the binding site, and which scoring function to use. The scoring function is of critical importance as it assesses the goodness of the fit, producing a quantitative score which can be used to rank each individual ligand. Many aspects are taken into consideration when evaluating the interaction of the protein with each ligand including steric fit, electrostatics, polar interactions and hydrogen bonding. The problem is compounded by the many possible conformations the ligand (or protein) takes on. The scoring function must evaluate each of these binding poses. Some of the major critiques of docking are the inability to calculate the free binding energy correctly (possibly because of the additive nature of most scoring functions), protein main-chain flexibility, the correct prediction of water in binding and the intensive computational resources required. In order to alleviate some of these issues, docking is sometimes used as a filtering first step before a more rigorous and computationally intensive molecular dynamics simulation. The top hits of the docking experiment are then rescored using MD. In a typical workflow, large virtual screening databases are first filtered using fast and inexpensive docking protocols. This rescoring is based on more physically realistic techniques for binding free energy estimations such as thermodynamic integration, free energy perturbation, linear interaction energy and molecular mechanics/Poisson-Boltzmann and surface area (MM/PB-SA). Overall, this provides a more accurate prediction of the binding affinity between the protein and the ligand (compared to the scoring function in docking tools). Computer-aided drug design is an active field of research, which has gained a lot of momentum in recent years - mostly driven by the decreasing productivity of the pharmaceutical industry to find new drugs.

5 Limitations of MD-based methods

The main force fields that are currently being employed for biomolecular simulations include AMBER (Asensio & Jimenez-Barbero, 1995), CHARMM (MacKerell et

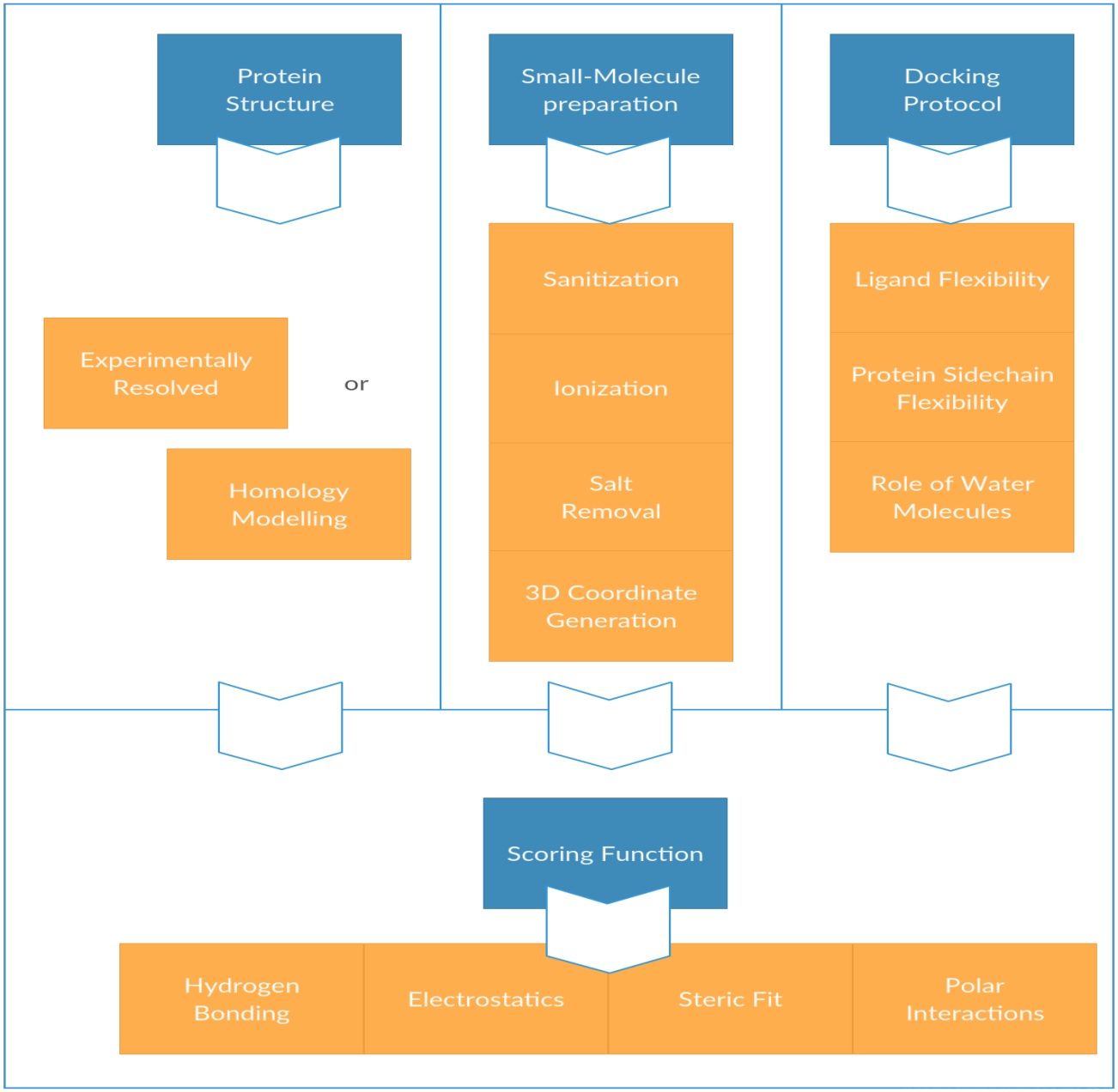

Figure 2: Components of a computational protein-ligand docking experiment. The goodness-of-fit of different small-molecules in a protein's pocket is assessed by means of a scoring function. The top-ranked results may serve as input to more computationally exhaustive techniques, such as Molecular Dynamics.

al., 1998), and OPLS (Jorgensen & Tirado-Rives, 1988). Although, extended parametrisation for amino acids, nucleic acids, lipids, carbohydrates, and several ionic species has been included in the parent force fields in recent years, the variability of small molecules (i.e., ligands) still poses a challenge to condensed-phase force fields. Thus, the user must carry out specific parametrisation. The latter is a time-consuming and an error-prone procedure, and has lead to the development of some general force field sets such as GAFF57 for AMBER, and CGenFF58 for CHARMM, together with specific parametrisation toolkits. Several challenges must be overcome to further increase the importance of MD-based methods on drug design. The molecular mechanics force fields that are presently available partially or fully neglect charge transfer and polarisation effects, as well as many electronic-based interactions. The current limits of force field and MD-based methods allow certain

target families, such as metalloproteins, to be studied with limited accuracy (De Vivo, Masetti, Bottegoni & Cavalli, 2016).

6 Conclusion

It is the combination of computational approaches that encompass techniques such as molecular dynamics simulations and docking, together with the interpretation of related experimental structural data, which is essential to provide a comprehensive understanding of the motions in proteins and their assemblies. Information on the latter is crucial when synthesising improved biomolecules and designing new drugs.

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Research Article

Geological, Geomorphological and Archaeological Sites in Pachino and Portopalo di Capo Passero Areas (Syracuse, Southeastern Sicily)

Pietro Carveni[1], Antonello Capodicasa[2], Antonio Iucolano[3], Patrizia Sibi[4] and Mario Valletta[*5]
[1] *Università di Catania, Italy*
[2] *Associazione Studi Storici e Culturali di Pachino, Italy*
[3] *Istituto Tecnico Superiore Archimede di Modica, Italy*
[4] *Università degli Studi della Tuscia, Italy*
[5] *The Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy.*

Abstract. The studied area corresponds to the extreme south eastern side of Sicily, characterized by the presence and coexistence of peculiar natural characteristics, connected to some expressions of a "virtuous" anthropic activity. Firstly, the purpose and objectives of the research are described. Characteristics of geological evolution, studied under structural, stratigraphical and geomorphological are later described. Some of the more important sites of geological, geomorphological and archaeological interest are also discussed.

Keywords: Geoarchaeology, Geomorphology, Pachino, Portopalo di Capo Passero, Tuna salt factories

1 Introduction

The extreme south eastern side of Sicily is characterized by the coexistence of several interesting sites, pertinent to geological, geomorphological and archaeological aspects that deserve greater acknowledgement.

This paper aims to take a census of sites with value of *Cultural Good* and to describe them. The requirement for this arises from the absolute need to protect, and use to its advantage, a rich heritage that is currently exposed to the risk of disappearance. This is due to anthropic foolish interferences and/or conscious and inconscious negligence of citizens and local managers.

Unfortunately, many environmental problems have already arisen, but if communities and local administrations were informed of the scientific, ecological and cultural aspects of this heritage many could have perhaps been prevented. Through the initiation of work that aims to preserve and recover this heritage, it will be possible to enrich and diversify the touristic opportunities the area offers, taking a meaningful step further towards a solid Sustainable Development.

The Geosites and Geomorphosites of Pachino and Portopalo of Capo Passero territories are the "consequence" of a long and complicated palaeogeographic evolution.

More interesting formations are thought to be a product of the effusive processes of the Cretaceous age and to the erosive action of the sea along the coasts. Remains of superficial eruptive structures, lined up along directrices of tectonic weakness, and many dykes with similar orientation, belong to effusive processes. In comparison, slopes, flat spaces of sea abrasion, marine terraces, marine caves, potholes and coastal ponds are connected to the erosive action of the sea. Complex shapes of karstic surfaces, epygeal and hypogeal, carbonatic rocks of the Cretaceous and Eocenic age, are also present.

The rise of sea level during historical ages is proven by the presence of archaeological sites and historical installations that are now partially or entirely submerged.

Morphological characteristics cannot be assigned to one scheme of evolution, due to the lithological variability of the outcropping rocks and their different exposure during geological time to atmospheric agents.

2 Geological – Structural frame

The basin of the central Mediterranean Sea, within Sicily, is characterized by the structural domains linked to the collision between the African and European continental plates. These plates have joined, giving rise to the Apenninic–Maghrebid Orogen, a corrugated belt which

Correspondence to: Mario Valletta (mariovalletta@iemest.eu)

forms the Apenninic Ridge. This belt passes through Sicily and the Straits of Sicily, and continues along the North African coasts of Maghreb.

In the north eastern sector of Sicily, the Apenninic–Maghrebid Orogen is formed by the presence of the Apenninic–Maghrebid Belt (Fig. 1: **cam**) and the Kabilo–Calabride Belt (Fig. 1: **ckc**).

The Apenninic–Maghrebid Belt is made by nappes with southern vergence, corrugation of which implicated sedimentary sequences of Tetide and of joined minor palaeodomains, originally placed between edges of European and African plates. The Kabilo–Calabride Belt is made by nappes of crystalline, with a basement origin involved in hercynic metamorphism, along with some components of primary meso-caenozoic sedimentary sequences (Finetti I, Lentini, Carbone, Catalano & Del Ben, 1996).

Nappes of Apenninic–Maghrebid Orogen overthrust Hyblaean foreland areas are regarded as a stablecomponent of African plate margin (Burollet, Mugnot & Sweeney, 1978). The sequence outcropping in the Hyblaean foreland is prevailingly formed by calcareous rocks. The age extends from Triassic to Middle Pleistocene. Cretaceous, Miocenic and Pleistocenic basic effusive rocks (connected to extensional tectonic phases) are embedded within sedimentary rocks (Cristofolini, 1966; Di Grande, 1967, 1969, 1972; Carbone, Grasso & Lentini, 1987; Amore, Carveni, Scribano & Sturiale, 1988; Carveni, Grasso, Romano & Tricomi, 1991; Carveni, Romano, Capodicasa & Tricomi, 1991; Carveni, Leonardi & Romeo, 1993; Carveni & Sturiale, 1999).

Hyblaean foreland borders on the east with the oceanic crust of the Ionian Basin (Finetti I, 1982) through Hyblaean-Maltese continental slope (Fig. 1: **SIM**), morphological expression of normal step faults system: their extension towards the north intersects the eastern side of Mount Etna (Cristofolini, Lentini, Patane' & Rasa', 1979).

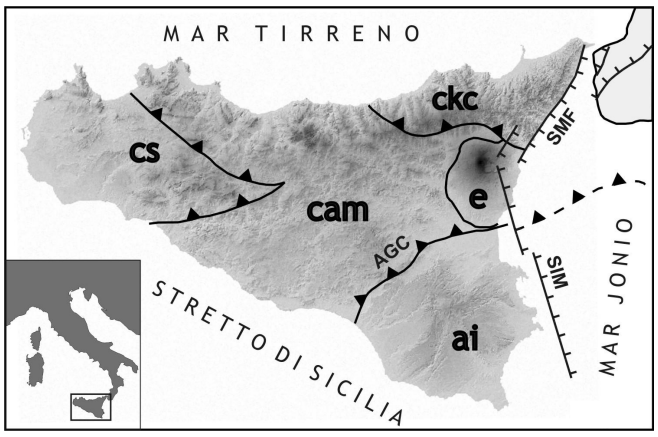

Figure 1: Structural scheme of the Sicily - **ai**) Hyblaean Foreland; **cam**) Apennine-Maghrebid Belt; **ckc**) Kabilo-Calabride Belt; **cs**) Sicana Belt; **e**) Etna volcano; **AGC**) Outcropping border of Gela-Catania foredeep; **SIM**) Hyblaean-Maltese continental slope fault system; **MF**) Messina-Fiumefreddo fault system (from Finetti I, Lentini, Carbone, Catalano & Del Ben, 1996).

3 Geology of the Pachino and Portopalo area of Capo Passero

The geological area studied coincides with the extreme south eastern part of Sicily and structurally, with Hyblaean foreland, is one of the most important structural elements of eastern Sicily. It is regarded as part of the African continental crust (thick more than 30 km), that would be actual continental shelf, contiguous to Apenninic–Maghbrebid Belt area, characterized by intense tectonic deformation (Barberi et al., 1974; Amodio Morelli et al., 1976).

Iblean foreland is delimited in the north west by the Caltanissetta Basin, an asymmetric trench, wedged between the foreland and the belt. The south eastern part of the formation constitutes the Gela-Catania foredeep. Off the eastern coast, Iblean foreland is cut off by the Iblean–Maltese slope, which separates the thin belt of continental platform and the Malta Channel from the Ionian Bathial plane, making up the western edge of all the Ionian Basin.

The studied area is part of the eastern sector of Hyblaean Cretaceous-Upper Miocenic geological sequence (Carbone, Grasso & Lentini, 1982), and is characterized by a basement of Cretaceous subaerial basic volcanites on which a sequence, prevailingly calcareous, is transgressive. There are many stratigraphic gaps ranging from Maastrichtian to Quaternary (Carveni, Romano et al., 1991).

The outcropping stratigraphic sequence is:

CRETACEOUS VOLCANITES (Fig. 2: **Cv**) – Very weathered basic volcanites outcrop widely between inhabited places of Pachino and Portopalo and are well observable along the cliff at northern of Portopalo. Hoffmann (1839) compared volcanites outcropping in the area of Pachino to Palagonitic Tuffs, a term denoting volcanic rocks typical of the Palagonia area, afterwards identified by Rittmann (1958, 1973) as volcanic rocks connected to submarine eruptions and named hyaloclastites. Hoffman's interpretation conditioned many researchers, who ascribed volcanic rocks of Pachino to submarine eruptions.

As a matter of fact, the rocks in question are subaerial lava flows with subordinate pyroclastic products (Carveni, Romano et al., 1991).

The Cretaceous age has been ascertained on the basis of the presence of Cretaceous calcilutites, with *Globotruncanae* discovered during drilling works (Colacicchi, 1963; Patacca, Scandone, Giunta & Liguori, 1979), as bedrock, and of Maastrichtian calcirudites with

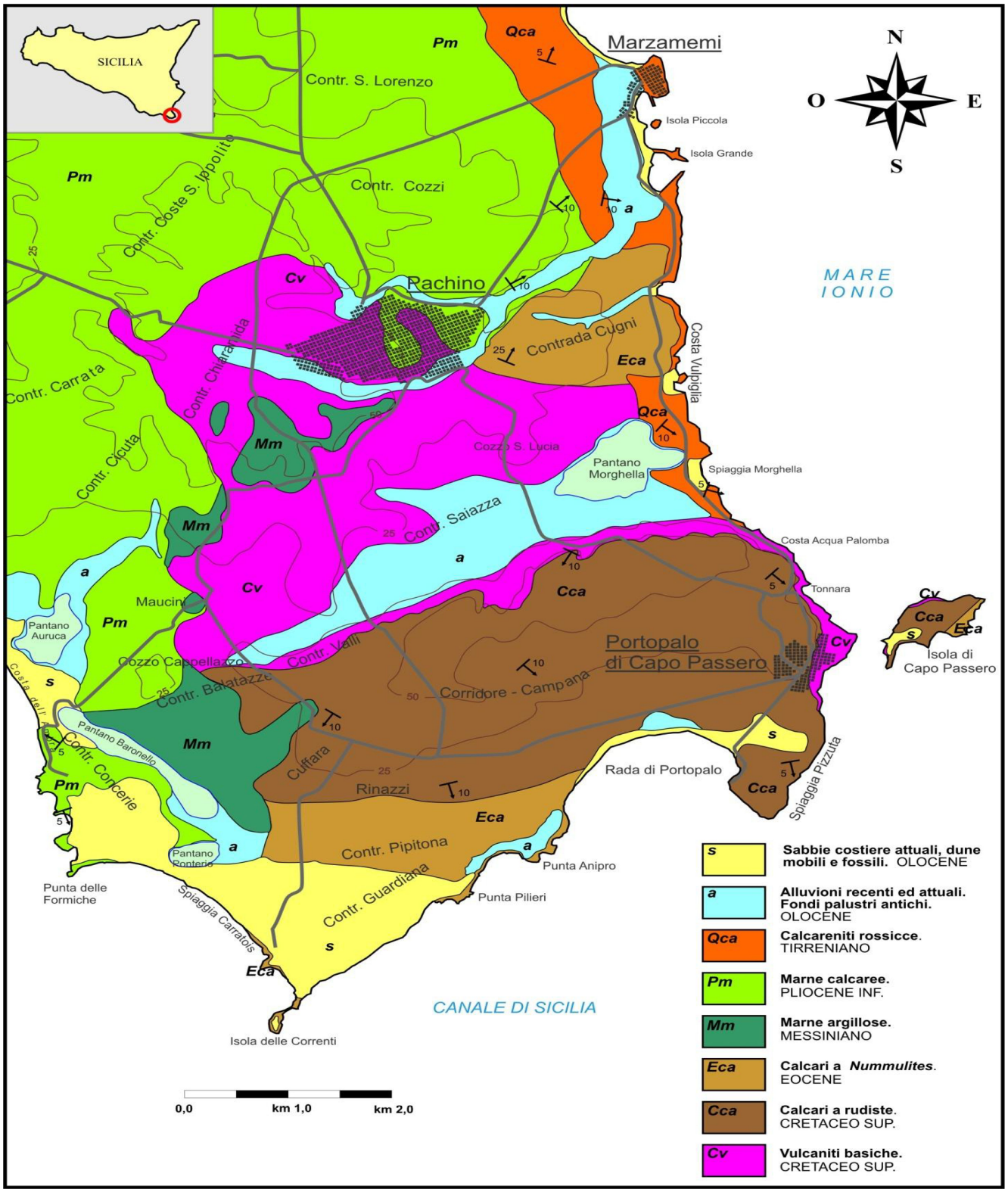

Figure 2: Geological map of Pachino and Portopalo area (from Carveni & Capodicasa, 2011). **s**) Present day beaches; dunes (Holocene); **a**) Recent and present day alluvial deposits; deposits of old marshy areas (Holocene); **Qca**) Reddish calcarenites (Tyrrhenian); **Pm**) Calcareous marls (Lower Pliocene); **Mm)** Clayey marls (Messinian); **Eca**) Limestones with Nummulites (Eocene); **Cca**) Calcirudites with Rudistae (Upper Cretaceous); **Cv**) Volcanic rocks (Upper Cretaceous).

Rudistae at the top (Colacicchi, 1963) as of radiometric (K/Ar) datations.

Carveni, Romano et al. (1991) identified, from the base to the top, the following formations:

ACQUA PALOMBA LAVA SERIES (Fig. 3: **12**) – they are mainly constituted by alkali-basalt lava and are clearly observable along the cliff at the east of Portopalo (Carveni et al., 1991 b). Several members of this series have been distinguished.

At the sea level, Porticciolo megaporphiric lavas outcrop. In the lower levels, they are strongly weathered, jointed and similar to volcanoclastic products if superficially analysed. At contact between a lava flow and an underlying one, there are clearly visible wall rocks, connected to the phenomena of thermometamorphism.

TONNARA OLIGOPHIRIC LAVAS – follow a clear surface of contact. They are rough and extremely weathered, with a reddish-purple blue colour. In particular, this aspect is connected with the way of emplacement that is the method of how lava pours into the sea. Lava flows can be very fluid single units of flow, prevailingly formed by spheroidal or long bodies (connected by thin strips, variously lying upon, of lava), modelling themselves on underlying surfaces.

The following effusive episode gave rise to PLAGIOCLASIOPHIRIC LAVA: some dykes of alimentation are recognizable along the Acqua Palomba cliff. These lavas are compact and characterized by a columnar structure and desquamation, like onions, in the upper levels.

PORPHIRIC LAVAS – with prevalence of crystals of augite are weathered, joined and outcrop above.

BASANITIC SUBVOLCANIC BODY (Carveni, Romano et al., 1991) – approximately 40 metres thick, and are formed by very compact grey rocks with associated pyroclastites of little/medium dimensions. These outcrop at the north of the Acqua Palomba spring. It is very likely to be the filling of an important eruptive joint.

In the areas studied, the census consists of more than 100 dykes. Among them, some have fed outcropping lava flows, whilst many others, which have crossed all the described volcanic series, located at the top, are broken off by erosional boundaries connected to the movement of swells. All of the lava flows, weathering and outcropping in the hinterland of Pachino and Portopalo, have been ascribed to the Acqua Palomba Series due to the impossibility of further subdivisions.

Other volcanites have been recognized and distinguished at the top. They are:

COZZO FILUA AND CONTRADE SAIAZZA AND TIGANELLO APHIRIC LOWER LAVAS (Fig. 3: **11**) – Aphiric lava flows with remarkable decimetric columnar structure and planes of flow lamination. They outcrop along the western slope of Cozzo Filua and in the countrysides of Tiganello, Saiazza and Chiusa di Pozzo. The bedrock has been assigned to members of the Acqua Palomba Series, although strong weathering makes it extremely difficult to identify.

CONTRADA MALTEMPO LAVAS AND PYROCLASTITES (Fig. 3: **10**) – Pyroclastites are formed by lavic fragments and weldered scoriaceous elements, sometimes cemented by secondary calcite. Outcrops of boulders and fragments of lava (cumulates basalts according to Carveni, Romano et al., 1991) are discontinuous. It is not possible to observe the contact between lavas and pyroclastites. The area of outcropping is at the northern area of Pantano Marghella. K/Ar dating of lavas gave an age of 80.0 ± 1.3 Ma (Carveni, Romano et al., 1991).

CONTRADA TIGANELLO PORPHIRIC LAVAS (Fig. 3: **9**) – This member of the sequence is composed of a series of little and thick lava outcrops, connected to a supposed area of linear emission, with a strike NE/SW. Lavas are compact with many small crystals of augite. The structure is porphiric. K/Ar dating is 79.9 ± 1.3 Ma (Carveni, Romano et al., 1991).

COZZO PAGLIARO PYROCLASTITES (Fig. 3: **8**) - These pyroclastites can be related to red-purple blue weldered small scoraceous elements, and assumed connected to the same effusive fracture that gave rise to porphiric lavas of Contrada Tiganello (Carveni, Romano et al., 1991).

COZZO SANTA LUCIA MEGAPHORPHIRIC LOWER FLOW AND COZZO FILUA UPPER FLOW (Fig. 3: **7**) – Cumulates basalts (Carveni, Romano et al., 1991), which outcrop around homonimous height, extend towards the south, with a total thickness of approximately 20 metres. An outcrop of similar lavas is present at the top of Cozzo Filua, above the aphiric lava unit.

COZZO SANTA LUCIA OLIGOPHORPHIRIC UPPER FLOW AND ASSOCIATED PYROCLASTITES (Fig. 3: **6**) – Porphiric alcali-basaltic lavas (Carveni, Romano et al., 1991), with evident columnar structure and a thickness of approximately 10 metres. Crystals of augite are prevalent, but less represented than those of olivina. A level of pyroclastites consisting of red weldered scoriaceous elements and rare volcanic bombs are also associated. K/Ar dating is 79.7 ± 1.3 Ma (Carveni, Romano et al., 1991).

4 Sedimentary rocks

At the end of Cretaceous volcanic activity, the area of Pachino and Portopalo was affected by a subsidence that caused a partial ingression of the sea. The Cretaceous, terrigenous and carbonatic deposits, at the top of Acqua Palomba Series volcanites, demonstrate a crucial change

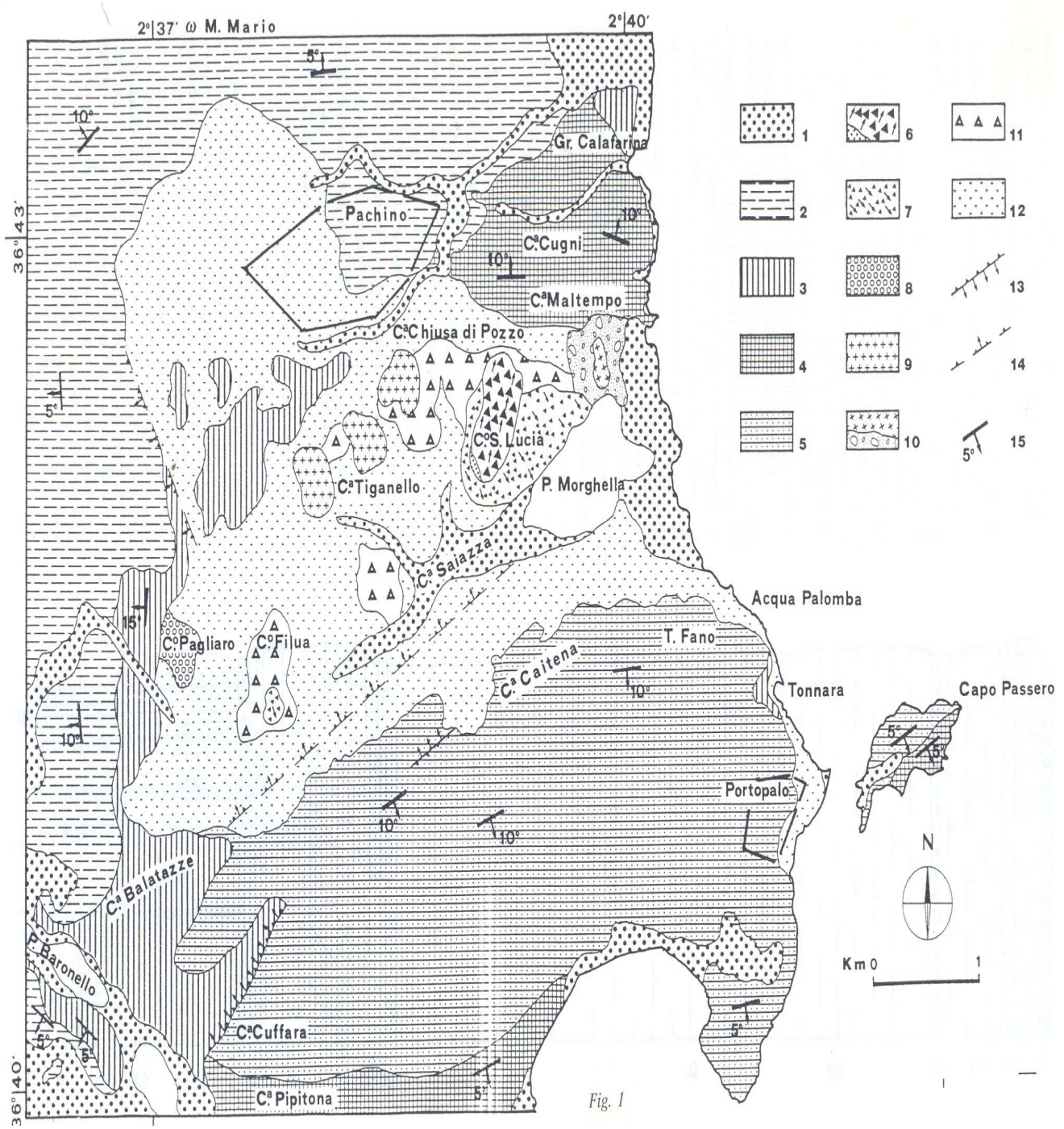

Figure 3: Geological scheme of area of Pachino and Portopalo of Capo Passero (Carveni, Romano, Capodicasa & Tricomi, 1991). **1**) Quaternary deposits (Calcarenites; Terraced beaches deposits; Hypogean deposits; Fossil dunes; Deposits of present day beaches); **2**) Pliocenic deposits (Trubi; Middlepliocenic conglomerates; Middlepliocenic marls); **3**) Miocenic deposits (Grotta Calafarina Calcarenites; Isola delle Correnti Breccia; Tortonian marls; Messinian marls); **4**) Eocenic deposits (Eocenic conglomerate; Cozzo Cugni Calcirudites; **5**) Cretaceous deposits (Contrada Càitena Sands; Isola di Capo Passero Conglomerates; Portopalo Calcirudites); **6**) Cozzo Santa Lucia Oligoporphiric upper flow and associated pyroclastites; **7**) Cozzo Santa Lucia Aphiric lower flow and Cozzo Filua upper flow; **8**) Cozzo Pagliara Pyroclastites; **9**) Contrada Tiganello Porphiric lavas; **10**) Contrada Maltempo Lavas and pyroclastites; **11**) Cozzo Filua and Contrade Saiazza and Tiganello Aphiric lower lavas; **12**) Acqua Palomba Lavas Series; **13**) Faults; **14**) Presumed faults; **15**) Dip of the strata.

in the geological evolution of this area.

The base of the Cretaceous sedimentary series is formed by yellow, thin, terrigenous deposits, referred to as the Contrada Caitena Sands (Carveni, Romano et al., 1991). This resulted from the erosion of underlying volcanic rocks. Eastward, sands pass to Isola di Capo Passero Conglomerate (Carveni, Romano et al., 1991), formed by decimetric spherical cobbles originating from the dismantlement of Cretaceous volcanic rocks. Interfingering between sands and conglomerate is not visible, but hypothesized on the basis of stratigraphic considerations, because Maastrichtian CALCIRUDITES WITH RUDISTAE (Colacicchi, 1963; Camoin & Duchafour, 1980; Matteucci, Schiavinotto, Sirna & Russo, 1982) (Fig. 2 **Cca**; Fig. 3: **5**) outcrop in conformity on the top of both lithotypes. Calcirutides are well developed on the top of alkaline basalts outcropping along the northern cliff of Isola di Capo Passero and along the coast at the east of Portopalo. Disconformity with underlying volcanites is emphasized by an erosional surface associated with the motion of the swells, under a low sounding depth. Truncation of many dykes makes this phenomenon even more evident.

Calcirudites are characterized by many and stratigraphically important fossil species: *Hippurites cornucopiae* De France, *Sabinia aff.* aniensis Parona, *Mitrocaprina bulgarica* Tzankov, *Hydnophoraraea spp.*, *Montastraea spp.*, *Actinastraea spp.*, *Columnastraea pachinensis* (De Gregorio), *Orbitoides apiculata* Schlumberger, *Omphalocyclus macroporus* (Lamarck), *Siderolites spp.*, *Simplorbites gensacicus* (Leymerie), *Hellenocyclina beotica* Reichel (Carbone et al., 1987).

LIMESTONES WITH NUMMULITES (Fig. 2: **Eca**; Fig. 3: **4**) – At the top of the Calcirudites with Rudistae outcrop, with angular unconformity, there are the limestones present with Nummulites. At the base there is a conglomeratic level: clasts derive from the erosion of aphiric lavas and, subordinately, of calcirudites with Rudistae (Trevisan, 1936; Colacicchi, 1963).

From a lithological point of view, it is a question of calcirudites and calcarenites and, locally, of micrites without fossils. Depositional environments can be referred to as a sea not too deep, with high energy and clear waters. Checchia Rispoli (1905b, 1905a) attributed these limestones to Eocene; Trevisan (1936) and Colacicchi (1963) specified attribution to Lutetian.

The colour is whitish-yellow, and there is generally little evidence of stratification.

There are many vughs and fossils containing internal models of Mollusca, Nummulites, fragments of skeletons of Corals and calcareous Algae (Carveni, Romano et al., 1991).

CALAFARINA CAVE AQUITANIAN CALCIRUDITES WITH LEPIDOCICLINAE – Sedimentation of these calcirudites occurred during the Aquitanian, in apparent conformity with Eocenic limestones with Nummulites outcropping at Cozzo Cugni (Fig. 3: **3**) (Colacicchi, 1963). They are assimilable to the member of Siracusa Limestones of Monti Climiti Formation (Lentini et al., 1986).

Limited extension of the outcrop and the noticeable lithological analogy with underlying Eocenic limestones impeded a long time (reinserted) the identification of these limestones, which was made by Colacicchi (1963). An outcrop of smaller dimensions, with reworked fossil fragments of Rudistae and Orbitoides, formed by biosparrudites and biolitites was identified by Amore et al. (1988) along the cliff at the north east of Portopalo.

TORTONIAN DEPOSITS – In the Isola delle Correnti, a breccia formed by calcareous fragments outcrop at the top of aquitanian calcirudites. According to Ruggieri (1959) and La Rosa (1974) this would be basal sediment of Tortonian transgression. Tortonian azure marls of the Tellàro Formation outcrop in conformity with breccia. Modest areal extension does not allow the determination of total thickness; however, data deriving from surroundings indicate a thickness of 70 metres (La Rosa, 1974). Microfauna, studied by Colacicchi and Romeo (1960), indicates the Tortonian age.

MESSINIAN ARGILLACEOUS MARLS – At the southwest of Pachino, along the northern side of Pantano Baronello, Messinian argillaceous marls (Fig. 2: **Mm**) studied by Colacicchi and Romeo (1960), outcrop in conformity with marls of Tellàro Formation. The contact is clear and underlined by a level of Ostrea coclear, with a thickness of a few centimetres.

The hill, on which the village of Pachino lies, is characterized by direct superposition of Messinian sediments on volcanic terms. On the contrary, at Contrada Balatazza and Contrada Cuffara, they are unconformed on Cretaceous and Eocenic limestones, making a marine transgression that occurred during the Messinian age evident. Strata are centimetric, with the prevalence of calcareous or argillaceous components. Total thickness, obtained from surroundings, is approximately 30 metres. Surroundings made evident even the presence of strata of gypsum, unknown in the studied area but outcropping a little to the north, at the top of marls (La Rosa, 1974).

TRUBI – The term Trubi indicates, in Sicily, a sequence characterized by marly limestones and calcareous marls, rich of Foraminifera and closely alternating. The colour is white and the fracture is typically conchoidal.

Trubi outcrop extensively in the north and west of Pachino (Fig. 2: **Pm**), strata are rarely clear and thickness is of 50–70 centimetres. Trubi pass, whether later-

ally or vertically, gradually to calcarenites. At the base, there is a conglomeratic poligenic bank, some metres thick and formed from pebbles, which are either rounded or rich in edges. These originate from the erosion of underlying Cretaceous and Eocenic carbonatic rocks (Piano Casa Nova). Microfaunistic associations are those of zones with Globorotalia margaritae and with Globorotalia puncticulata (Lentini et al., 1984). Among the few macrofossils, there is Liostrea coclear. On the basis of surrounding data, thickness is approximately 50 metres (La Rosa, 1974). The age of sedimentation is lower Pliocene.

QUATERNARY REDDISH CALCARENITES – Along the eastern coast of the studied area, reddish calcarenites (Fig. 2: **Qca**), which are well cemented and with granulometry from middle to fine, outcrop (Fig. 3: **9**).

Fossils do not allow certain dating. Trevisan (1936), Ruggieri (1959), Colacicchi (1963) attributed a probable Tirrhenian age on the basis of considerations about height and subhorizontal layering.

The particular importance of calcarenites is attributed to use of that rock as building materials during the Greek-Roman period.

SCALO MANDRIA CONGLOMERATES – Near the village of Portopalo, along the coast of Scalo Mandria, there are two little outcrops of beach deposits, resting on Cretaceous lavas and placed at different heights.

The first is made from a small, cemented conglomerate with centimetric pebbles, prevailingly volcanic and subordinately calcareous into a reddish sandy matrix. The height of the base is 2.30 metres above sea level.

The second, outcropping a few metres southward, is made from a well-cemented conglomerate with decimetric pebbles, prevailingly calcareous and subordinately volcanic, into a whitish matrix. The height of the base is 1.20 metres above sea level.

The age of those conglomerates can probably be referred to as Quaternary.

HYPOGEAN FOSSILIFEROUS DEPOSITS OF ISOLA DI CAPO PASSERO – Along the northern coast of Isola di Capo Passero, bones of terrestrial mammalia, and blocks of carbonatic rock in yellow ochre mould were found. Deposition of mould occured around stalagmites (Carveni, Romano et al., 1991). It is an important fossiliferous layer, formed into a karstic cavity, where carcasses of entrapped animals accumulated within.

RECENT BEACH DEPOSITS – Along the beach before Isola di Capo Passero, there is a beach deposit formed from strata of sand and gravel, rich in fragments of fictile material and bones of tuna.

The outcrop is joined to a very important archaeological site: the height, which is approximately one metre above actual sea level, witnesses a recent relative sinking of the sea level.

FOSSIL DUNES – Cemented deposits of aeolian origin are present near the beaches.

PRESENT DAY BEACH DEPOSITS – Present along some of the coast, and are prevailingly made up of sands; more extended beach is that in front of Isola delle Correnti.

MOBILE DUNES – Mobile dunes are generally associated with sandy coastal deposits, from which originates material of alimentation.

ACTUAL ALLUVIAL DEPOSITS - Present along the streams, and are made up from gravels, sands and muds.

5 Geomorphological frame

As already accounted, morphological general configuration and morphological characteristics of the analysed territories cannot be related to a single evolutive scheme, due to the lithological variability of outcropping rocks and their different exposure to atmospheric agents during geological time. During geologic eras, many submersions and emersions of the studied area occurred. Consequently, reciprocal actions of sea abrasion and of superficial factors of erosion repeated, sometimes in association with some tectonic displacements. Such phenomena contributed greatly to the determination of different morphoevolutive processes and consequent different aspects of the landforms and landscapes.

The northern sector, characterized prevailingly by marly rocks, is marked by a series of small hilly raisings with a rounded outline, separated by large valleys. Erosional processes, mainly connected to the action of waters of surface run-off, are extremely contained, owing to little extension of drainage basins.

In the central sector, outcrop wide volcanic rocks from which emerge, as little and isolated hills, eruptive volcanic structures of the Cretaceous complex that are deeply eroded. As a whole, that area is a wide valley that, in its terminal part, enlarges to form coastal quagmire of Marghella. Hydrographic net is represented by short torrents, locally named *saie*, where downflowing of which happens only on occasions of particularly abundant and prolonged rains.

The southern sector is characterized by a monocline descending gently towards the south, delimitated at the north and east by retreated fault scarps. In this sector, it is possible to observe typical tabular morphology of calcareous landscapes, characterized by a dip of the strata, relatively uniform, and by a small inclination. Superficial erosive action is moderate, owing to the tenacity of outcropping rocks and their high draining power.

Littoral areas are characterized by low and rocky coasts, high cliffs perpendicularly on the sea and creeks, which are sometimes very narrow and deeply incised, or sometimes large and occupied by sandy deposits.

Behind some beaches there are little dunes not yet destroyed by agricultural training and by interventions of urbanisation, which, in the last period of ten years, changed the natural equilibrium of the coasts without the possibility of being recovered.

Little altimetrical difference between more high height of reliefs and base level (10–15 metres) is the cause of a sudden diminution of the velocity of waters along slopes, determining a phase of sedimentation after a very brief stage of transport.

The coastal inland is bordered by many ponds, representing the emergence of phreatic water tables. These occupy morphological hollows that sandy and/or calcarenitic bars divide from the sea.

The climate is Mediterranean arid, with long periods of nearly fully dry (from May to October), sporadically interrupted by precipitations with characteristics of storm, and months with temperate climate, characterized by brief rainy periods alternating with long periods of insolation.

6 Description of sites

GEOSITES AND GEOMORPHOSITES OF VOLCANIC ORIGIN – The origin of most important Geosites and Geomorphosites is volcanic. They characterize the Acqua Palomba cliff, where marine erosion made evident the internal structure of Cretaceous volcanic structures.

TORRE FANO NECK – A subvolcanic body, outcropping along the cliff in correspondence of ruins of Torre Fano. At petrographic analysis, it resulted to be a basanite (Carveni, Romano et al., 1991). The neck has been partially dismantled by marine erosion, which allowed an important eruptive apparatus to individuate.

ACQUA PALOMBA CLIFF DYKES – Along the Acqua Palomba cliff, a census of 111 dykes has been performed. Strike is comprehended between N 5° E and N 90° E, with a prevalence in the interval N 40°–50° E. Some dykes resist meteoric erosion better than walls, putting out on the plane of the field, while others are attacked more easily by marine abrasion, along with the formation of deep caves. Of particular interst is the zigzag pattern of the dykes. Such a phenomenon is attributed to be the wall of a columnar basalt, which determined the form of intruded mass.

GEOMORPHOSITES OF MARINE ORIGIN These Geomorphosites are often indicative of variations of the level of the sea. This is one of the reasons why they are so important.

CAVES EXCAVATED INTO THE DYKES – Phenomena of marine abrasion, as aforementioned, are particularly evident where dykes of a large thickness outcrop dykes that cross cretaceous volcanites.

CAVES ALONG NORTHERN CLIFF OF ISOLA DI CAPO PASSERO – The northern coast of Isola di Capo Passero is also affected by considerable erosive phenomena, giving rise to a cliff approximately 20 metres high. Action of the swells and local structural and lithological characteristics determined the morphological evolution of classic wave-cut notches into caves of remarkable dimensions.

CAVES UNDER CASTELLO TAFURI – At the north of the countryside of Portopalo, under Castello Tafuri, there are two caves connected to marine abrasion. Both develop into Cretaceous limestones, forming a rocky slope on which lies the castle, in correspondence to a small plane at the height of 15 metres above sea level. Research carried out does not supply useful elements for the determination of the age of their formation. Still, their height suggests a correlation with marine terraces of upper Pleistocene which, in the south eastern Iblean area, according to Carbone, Di Geronimo, Grasso, Iozzia and Lentini (1982), are present at the height of approximately 15 metres above sea level.

CORRUGGI CAVE – At the northeast of Pantano Marghella, there is a marine abrasion cave, known as the Corruggi Cave, before which, at a height of about 4 metres, is a plane. The cave, which comprises Eocenic limestones, is observed in archaeological literature, due to the discovery of manufactureted goods of final phase of upper Palaeolitic (Bernabo' Brea, 1949).

POTHOLES ON ISOLA DI CAPO PASSERO In the south eastern sector of Isola di Capo Passero, at the height of about 6 metres above sea level, there are several potholes near the border of the cliff. These caves concern Eocenic limestones. One of them, in particular, preserves inside rounded big calcareous blocks. Their vortical movement, connected to the breakers, excavated the rock. It is not possible to determine the age of formation of these morphosculptures. Their ubication, however, is testimony to the relative lowering of sea level.

POTHOLES OF SPIAGGIA CARRATOIS – At the north west of Isola delle Correnti, along the low rocky coast and at a height between 1.20 metres and sea level, there are many potholes comprising of algal Eocenic limestones. The caves of various dimensions are cylindrical. Walls are vertical and deep until 1.50 metres and the bottom is flat. Often, there are many caves blended to form big basins arriving at 7–8 square metres. Other potholes are present under sea level and are still active.

ABRASION PLANE OF SPIAGGIA PIZZUTA – At the south of the countryside of

Portopalo di Capo Passero, along the rocky coast denominated Spiaggia Pizzuta, it is possible to observe a marine abrasion plane comprising of Cretaceous calcarenites. The plane, which declines from a height of about 6 metres to sea level and cuts very evidently calcareous strata, witnesses a recent lowering of sea level.

7 Archaeological witnesses of sea level variations

NEOLITHIC SETTLEMENT OF SPIAGGIA MORGHELLA – Close to the eastern part of Pantano Morghella, archaeological excavations carried out at the beginning of '90s by Soprintendenza of Siracusa individuated rests of a wide coasting village of the neolithic age (Guzzardi & Basile, 1996). The southern sector of the installation is partially under sea level, but there are no studies useful in determining the rate of heightening of sea level.

OLD CANAL OF ALIMENTATION OF SALTERN OF MORGHELLA - A rectangular canal, deep at 2.90 metres and cut into Quaternary Reddish Calcarenites. Its function was to allow seawater to enter into Pantano Morghella, and was perhaps used as a saltern during the Greek-Roman period (Lena, Basile & Di Stefano, 1988). The canal is nearly entirely full of sand and is visible only in proximity of the area where sea waves break, it can be followed along some tens of metres towards the open sea. The upper border of the canal reaches a depth of only 1.40 metres at the point furthest from the coast. Calcarenitic level crossed by old excavation preserves tracks of old quarries of stones ("Latomie") of Ellenistic period, most of them submerged.

OLD MARZAMEMI QUARRIES OF STONES ("LATOMIE") – Wide old quarries ("Latomie") of the Greek period consist of Quaternary Reddish Calcarenites outcropping along the rocky coast of Marzamemi. From those quarries, probably going back to the fifth century before Christ (Guzzardi & Basile, 1996), blocks of calcarenite were extracted, many of which are still in place. "Latomie" are developed at various levels, with many of them totally submerged. Other little quarries of calcarenite, probably of the same period and partially submerged, are present on Isola Grande, Isola Piccola and the south of Spiaggia Morghella.

At Marzamemi, measurements carried out through subaqueous immersions pointed out that the lowest level of extraction is actually at 1.40 metres below sea level.

INDUSTRY FOR THE CONSERVATIONS OF THE TUNA OF SCALO MANDRIA – In front of Isola di Capo Passero outcrop rests an installation, of the Ellenistic-Roman period, where the manufacture of fish can be found. It is composed of basins excavated into lavic rock and utilized for the salting of tunnies from the fifth century before Christ to fourth century after Christ (Guzzardi & Basile, 1996). Basins were identified by a strong heavy sea during the 1981 winter (Bacci, 1983), and some of them, buried by the sand, are actually in correspondence of the area where sea waves break.

Unfortunately, available data does not allow the measurement of the amplitude of the phenomenon of submersion of archaeological sites. In spite of this, the presence of manufactured goods under sea level and disguised by the sand is clearly indicative of a sensible rise of sea level.

Recent deposits of bones of tunnies are to be regarded as discarded matter of manufacture of that industry.

BASINS OF CONTRADA CONCERIE – Along the coast at the north of Punta delle Formiche, there is a site of archaeological interest. There are some basins of Greek-Roman period, excavated into infrapliocenic calcareous marls, which are assumed to have been utilized for breeding fish. The basins are rectangular and are partially submerged. Measurements revealed the greatest depths of approximately 1 metre below actual sea level. Practical considerations allow us to presume that rising of sea level could have reached two metres.

8 Conclusion

Multiform peculiarities, characteristic of area of Pachino and Portopalo di Capo Passero, make the importance and value of that Cultural Heritage extremely evident.

These peculiarities identifiable in geological history are very fascinating. This area shows an element of specific importance in the sphere of evolution of the Mediterranean area; with the presence of palaeovolcanic phenomena. Indeed, deep explorations carried out to research petroleum and natural gas revealed the presence of underground volcanic rocks of Triassic age. Moreover, this area was particularly active during Cretaceous times; as the "interference" with coeval marine sedimentation; with a stratigraphic sequence of marine deposits getting untied until to the Tyrrhenian clearly show.

Geomorphological characteristics have the same interest. Morphotypes are specific and expressive answers manifold geological events and to characteristics of lithotypes.

In conclusion, the described sites have been the result of a peculiar geological history, of geomorphological evolution, and human activities.

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Research Article

An Updated Flora of Selmunett (St. Paul's Island) including Mosses and Lichens

Stephen Mifsud*[1], Edwin Lanfranco[2], Jennifer Fiorentino[3] and Stephan D. Mifsud[4]
[1]*Independent Taxonomist and Researcher*
[2]*Department of Biology and Institute of Earth Systems, University of Malta*
[3]*Department of Biology, University Junior College, Msida, Malta*
[4]*Independent Moss Researcher*

Abstract. A survey of four visits in Selmunett (Gżejjer ta' San Pawl) resulted in a number of new records comprising 23 lichens, 2 mosses and 20 higher plants. Five of these species are protected, amongst which *Plocama calabrica* is very rare whereas *Parietaria cretica*, a critical species only recorded from Selmunett in the Maltese islands, has been rediscovered after not being sighted for about 15 years and was suspected of having become extinct. Records resulting from this survey are compared with those from previous records or surveys between 1927 and 2012. These are examined critically, identifying possible misidentifications as well as establishing their status, thus producing a final update of the florula of Selmunett.

Keywords: Selmunett, St. Paul's Island, Flora of Malta, *Parietaria cretica*

1 Introduction

Selmunett, also known as Saint Paul's Island, il-Gżejjer ta' San Pawl and, in the distant past, as Ta' Barba Marku, is situated in the north east of mainland Malta, isolated by about 100 m of shallow water. The recent name is derived from the belief that the shipwreck of Saint Paul took place in the whereabouts of this islet (Farrugia Randon, 1995).

At its central part, the islet has a shallow isthmus about 100 m long which, in stormy weather, may become momentarily submerged by 'high water' and giving the inaccurate impression that it consists of two small islets. The length of the island is about 885 m and its widest part is about 200 m across. It consists of Upper Coralline Limestone, reaching up to 22–24 m above sea level at the south-western side. It has been uninhabited since the beginning of World War II. Earlier, a farmer used to live and raise crops in a number of fields. Rubble walls are still in relatively good shape, but an old farmhouse has largely collapsed.

The island features three main type of habitats: shallow littoral rocky ground exposed to sea spray, especially dominant in the eastern part and hence the smaller 'islet', which is only about 8 m above sea level; very degraded garigue turning into steppe in the abandoned agricultural areas covering much of the larger 'islet'; and low garigue remnants encircling most of the larger 'islet' and in the north and west. The island also features a small blue clay formation in the west but it does not sustain any plant communities typical of clay habitats. The cliffs are not high enough to support true chasmophytic or rupestral communities and there is no sandy shore or vegetated temporary freshwater rock pools.

Two main soil types have been reported. Terrarossa soil which is found dominating the smaller 'islet' and a mixture of Terrarossa and Xerorendzina in the larger 'islet'. As expected for a small islet, Selmunett has no surface water and its vegetation thrives only on rain water which percolates through fissures in a lower water table above the Blue clay stratum (Lanfranco, 1983).

The vegetation has to withstand harsh environment conditions, namely sea spray due its proximity to the sea and low altitude of the islet; strong winds and storms due to full exposure of the land; and a completely dry summer lasting for about four months. For this reason most of the plants are halophytes and/or xerophytes, with some more variety on the upper parts of the larger 'islet'.

**Correspondence to*: Stephen Mifsud (info@maltawildplants.com)

2 History of the flora of Selmunett

The first floristic inventory of this islet can be compiled from the records of Borg (1927); consisting of a total of 23 species. A series of excursions took place between 13th May 1973 to 30th May 1982, organised by the Natural History Society of Malta and initiated by Guido Lanfranco. Findings of these excursions were published in a dedicated issue of Potamon resulting in 80 species (Lanfranco, 1983), of which a major part were recorded previously by Lanfranco (1973). Sporadic visits took place later (e.g. Schembri & Lanfranco, 1983) but important contributions were visits, which were carried out by Lanfranco in 1990 and 1995, increasing the inventory by 30 and then 4 species respectively (Lanfranco, 1990, 1995). Farrugia Randon (1995) lists some previously recorded and rather common species on the islet. In two excursions organised by the Environment Protection Department, one in 24th March 2000 and one in 19th May 2004, 114 species, of which 20 were new records, were found in the first visits, and further three new species where recorded in the second (Stevens, 2000, 2004). The last survey was carried out by J. Sciberras, Sciberras and Pisani (2012) recorded 89 species of which 21 species were new additions and in majority were perennials (including phanerophytes and geophytes).

3 Methods & Material

Four site visits were planned to be spread once every three months (October, January, April and June) between 2014 and 2015, but due to rough seas persisting in winter, the four site visits took place on 18th October 2014, 14th March 2015, 2nd May 2015 and 19th June 2015. Stephen Mifsud [SM] and Edwin Lanfranco [EL] participated in all four visits, whereas Jennifer Fiorentino [JF] and Stephan D. Mifsud [SM2] joined in the third visit and studied lichens and mosses respectively. MEPA endorsed the required permits to visit the islet which is designated as a protected site. The species encountered were recorded and a rough frequency on the islet was estimated by visual inspection. Frequency is reported in Table 1 using a scale of 5 levels as follows:

- **4** common and abundant throughout most areas of the islet;
- **3** frequent or locally frequent in rather dense populations;
- **2** scarce or infrequent;
- **1** rare and occasional;
- + very rare, just few individual plants.

All species were photographed for future reference, while sampling of a few species (esp. lichens) for identification purposes was carried out. Specimen samples of mosses and vascular plants were discarded after their identification was carried out, while those of lichens were kept at the private herbarium of [JF]. Results are displayed in Table 1 comprising a list of species recorded in our four visits, including their frequency and current protection status. Table 2 consists of a log of all plant species recorded in previous visits from Borg (1927) to J. Sciberras et al. (2012) using the original cited taxon. Finally, species recorded or reported in previous visits and not found during the visits covered by this study are given in Table 3. The last record, and number of times it had been recorded for each species is also included in this table.

Some problematic groups of taxa are treated here in a wide sense: these are the *Plantago coronopus* complex; the *Allium ampeloprasum* group and the problematic *Daucus carota* s.l. and *D. gingidium* s.l., all of which require further investigation to determine their taxonomic status on the Maltese islands. One of us [SM], together with Owen Mifsud, are currently investigating the *Allium ampeloprasum* group. Our taxonomic treatments and further notes on the status and distribution of the flora of Selmunett are dealt in detail further below.

The nomenclature of some previously recorded taxa, mostly in Borg (1927) or/and Lanfranco (1983), has been updated to their current classification according to (The Plant List, 2013) and hence are not included in Tables 1 and 3 in order to avoid statistical confusion and duplication of taxa which refer to the same species. These taxa are: *Senecio cineraria* DC. *sensu* Borg (1927) and *Senecio bicolor* (Willd.) Tod. (*sensu* various authors) the records of which are now replaced by the recently described *Jacobaea maritima* subsp. *sicula* (Willd.) B.Nord. & Greuter; *Jasonia glutinosa* (L.) DC. *sensu* Borg (1927) is replaced by *Jasonia bocconei* (Brullo) M.Pardo & R.Morales; *Inula crithmioides* L. is replaced by *Limbarda crithmioides* (L.) Dumort; *Statice minuta* var.-*virgata* W.-St. *cordata* Desf. non L. *sensu* Borg (1927) and *Limonium oleifolium* Mill. *sensu* Lanfranco (1983) are replaced by *Limonium virgatum* (Willd.) Fourr.; *Statice minuta* var. *reticulata* Rchb. is replaced by *Limonium zeraphae* Brullo.

4 Results

A total of 140 species of vascular flora were recorded by [SM] and [EL] in the four visits. 27 lichens were identified by [JF] and 3 moss species by [SM2] from the visit in May 2015 (refer to Table 1). About 200 higher plants have been recorded during the last 90 years (refer to Table 2) but, as mentioned above, the identity of few species is questionable, others, namely those which were reported once, may be considered as short-lived casuals or accidental introductions, while those species that have not been observed for several decades can now be presumed extinct from Selmunett (refer to Table 3).

Our visits resulted in 20 new records of vascular plants, of which some are protected or listed in the red

data book for the Maltese islands (Lanfranco, 1989). First visit: *Adiantum capillus-veneris* L., *Prospero autumnalis* (L.) Speta, *Hyparrhenia hirta* (L.) Stapf, ***Plocama calabrica*** (L.f.) M. Backlund & Thulin (RDB, strictly protected; Fig. 1), *Pistacia lentiscus* L. (protected); second visit: *Hippocrepis biflora* Spreng., *Lotus tetragonolobus* L., *Medicago lupulina* L., *Gladiolus* sp., *Ophrys bombyliflora* Link, *Galium murale* (L.) All.; third visit: *Ornithogalum narbonense* L., *Carlina gummifera* (L.) Less., *Sagina maritima* G.Don, *Spergularia diandra* (Guss.) Heldr., ***Sedum litoreum*** Guss. (RDB, strictly protected; Fig. 1), ***Orobanche cernua*** Loefl. (RDB; Fig. 1), *Orobanche* cf. *minor* Sm., *Orobanche pubescens* d'Urv; fourth visit: *Heliotropium europaeum* L.

About 55 species, which have been recorded previously, were not observed in our four visits (Table 3). Several explanations can be postulated for many of these species. Some small or inconspicuous plants may have been overlooked (e.g. *Senecio pygmaeus* DC., *Frankenia pulverulenta* L., *Geranium molle* L., *Avena hirtula* Lag. and *Catapodium rigidum* (L.) C.E.Hubb.); others with a short flowering period may have been missed because of the timing of the visits, e.g. small plants which flower between December and March, during which we couldn't visit the islet due to rough seas (e.g. flowering *Romulea* spp., *Orchis collina* Banks & Sol. ex Russell and *Bellis annua* L.); other old records were likely short-lived casuals (e.g. *Fumaria officinalis* L., possibly disappeared following the abandonment of agriculture, *Trifolium tomentosum* L. and *Hyoscyamus albus* L.), whereas some reported taxa are likely cases of misidentification (*Sagina apetala* Ard. may have been a misidentification for *S. maritima* G.Don; *Parapholis filiformis* (Roth) C.E.Hubb. certainly mixed up with *P. invurva* (L.) C.E.Hubb. since this species is tied to saline marshes, a habitat which does not exist at Selmunett; *Geranium rotundifolium* L. confused with *G. molle* L. and *Salsola melitensis* Botsch. [= *Darniella melitensis* (Botsch.) Brullo] might have actually been *Arthrocnemum macrostachyum* (Moric.) K.Koch, if its previous identity was based on observations from a distance. However, some native species might have become extinct (refer to Table 3) such as *Thymbra capitata* (L.) Cav., recorded by Borg (1927) and never substantiated again or *Centaurea melitensis* L. and *Tordylium apulum* L. only recorded by Lanfranco (1973) and Mario Gauci (Haslam, Sell & Wolseley, 1977) respectively.

It is curious that a number of conspicuous native species, especially geophytes or phanerophytes, were not observed in our thourough surveys. Most important being *Pancratium maritimum* L., *Chamaerops humilis* L., *Scilla sicula* Tineo ex Guss., *Matthiola incana* subsp. *melitensis* Brullo, Lanfranco, Pavone & Ronsisvalle, *Halimione portulacoides* (L.) Aellen, *Iris sicula* Tod., *Anacamptis urvilleana* Sommier & Caruana, *Olea europaea* L. (J. Sciberras et al., 2012); *Orchis coriophora* L. [= *O. fragrans* Pollini] (Lanfranco, 1973; Stevens, 2000) and *Stipa capensis* Thunb. (Lanfranco, 1990; Stevens, 2000, 2004), the latter being recorded thrice.

It is strange that a number of perennials, including geophytes (see Table 3), which were recorded by J. Sciberras et al. (2012) only two years before our visits, were not found by us. The confirmation of their continued existence on Selmunett is important since some of them are protected or/and have RDB status, mostly for their rarity on the Maltese islands. Correspondence with one of the authors in J. Sciberras et al. (2012) was made to shed some light. The answer given was that most of the afore-mentioned species were found as seedlings and plantlets, which probably did not survive the following Summer. When asked for photographs, only seedlings of two *Matthiola incana* (L.) R.Br. s.l. (identified as subsp. *melitensis* by these authors) and a single juvenile plant of *Hyoseris frutescens* Brullo & Pavone was available from their end. The status of these species on Selmunett is currently uncertain and their establishment would be important because most of them has a threatened status for the Malta.

Flora Melitensis Nova (Sommier & Caruana Gatto, 1915) is the first publication which has a section and a short inventory on the local records of lichens. None of the lichens listed in this publication have Selmunett as locality, and the first records of lichens from Selmunett are only four species given by Lanfranco (1983). Consequently 23 lichens found during a brief survey in May 2015 and being listed in Table 1, may well be considered as first records for Selmunett. The detailed study of Maltese lichens has not been seriously undertaken until recent years.

Moss species recorded on Selmunett previous to this study were *Barbula unguiculata* Hedw. and *Tortella flavovirens* (Bruch) Broth. (S. D. Mifsud, 2012). Three species of mosses identified by one of us [SM2] during the present study are *Tortella flavovirens* (Bruch) Broth., *Entosthodon pulchellus* (H. Philib.) Brugués, and *Trichostomum brachydontium* Bruch, hence the latter two are new records for the islet. The search for mosses, scheduled on April 2015, was delayed by a few weeks due to rough weather, when Selmunett was dry and not favourable to study mosses.

Figure 1: Some important vascular plants from Selmunett: A. *Parietaria cretica* (14-Mar-2015); B. *Orobanche cernua* (2-May-2015); C. *Linaria pseudolaxiflora* (14-Mar-2015); D. *Plocama (=Putoria) calabrica* (14-Mar-2015); E. *Sedum litoreum* (2-May-2015); F. Inaccessible ledge at the western coast of the islet dominated by shrubs of *Arthrocnemum macrostachyum*. Salsola *(=Darniella) melitensis* was not observed in this area or any part of Selmunett. Photographs by Stephen Mifsud.

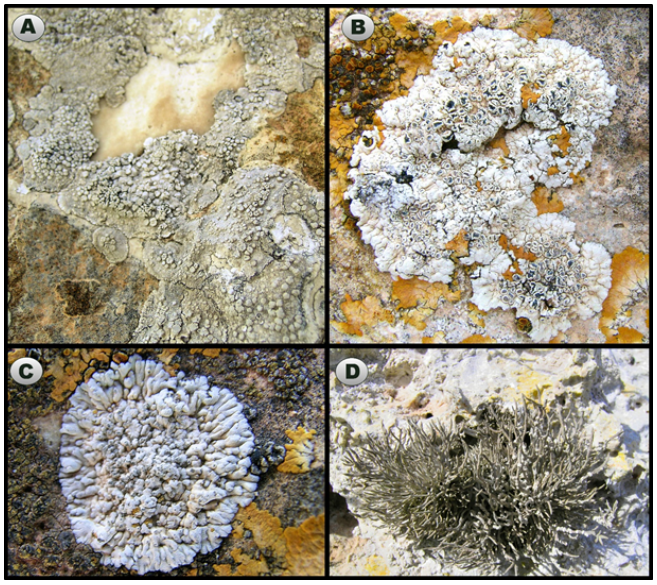

Figure 2: Some lichens from Selmunett: A. *Dirina massiliensis* (Schreb.) Norman; B. *Protoparmeliopsis (=Lecanora) muralis* (Schreb.) M. Choisy; C. *Lecanora lisbonensis* Samp.; D. *Roccella phycopsis* Ach. Photographs by Jennifer Fiorentino (2-May-2015).

4.1 Notes on some species, taxonomic updates and doubtful records

1. *Mesembryanthemum nodiflorum* L. is reported in old accounts to be dominant (Lanfranco, 1983), but in our visits we have found this halophyte to be scarce, almost absent, except for a large patch near the derelict farmhouse, at the larger 'islet', and infrequent to locally frequent in small pockets on the smaller 'islet'.
2. The shrubby endemic *Salsola melitensis* Botsch (= *Darniella melitensis*) (Botsch.) Brullo has been reported a few times from the islet (ref). This species was not found in our four visits. In order to carry out a thorough check, a boat trip around the entire islet and as close as possible to its shore was carried out during the fourth visit in an attempt to locate the species in inaccessible sites, especially at the northern and western cliffs. Powerful binoculars were used. All dominating vegetation sighted corresponded to *Arthrocnemum macrostachyum* (Moric.) K.Koch and *Limbarda crithmoides* (L.) Dumort. (refer to Fig. 1F), both large succulent shrubs, the former with inconspicuous flowers, the latter without flowers in late winter and spring, hence from a distance such as over the cliff top, may superficially look like *Salsola melitensis*. The status of this shrub on the islet is now uncertain from our observations, but it is reasonably possible that it was mistaken with large shrubs of *A. macrostachyum* located at an inaccessible ledge on the west.
3. The records of *Pancratium maritimum* L. and *Halimione portulacoides* (L.) Aellen are interesting because no sand dune or saline marshes occur in Selmunett. They were only recorded once (J. Sciberras et al., 2012) and after personal communication with the main author (*op. cit.*), we learnt that these and first records of other perennial plants were observed as seedling or young plants. Hence, these can be considered as cases of accidental germination or misidentification due to their young, immature state.
4. The *Allium ampeloprasum* L. complex in Malta is currently being studied by one of us [SM] and Owen Mifsud. Traditionally, *A. commutatum*, *A. ampeloprasum* and *A. melitense* (endemic) have been reported.
5. *Daucus rupestis* Guss. was previously recorded from Selmunett when little knowledge on this taxon was available. Its status in the Maltese islands needs further investigation, mainly from the fact that the description of the protologue makes reference to a much smaller holotype. As a result we could only discriminate between typical *D. carota* L. and *D. gingidium* L. s.l. in our survey.
6. The opinion of one of us (Lanfranco, 1983) was that *Ridolfia segetum* (L.) Moris was misidentified by Borg (1927) perhaps with stunted specimens of *Foeniculum vulgare* Mill. While this can be the case, one also has to consider that this species may have occurred in agricultural land, and since agriculture has been abandoned before the Second World War II (Farrugia Randon, 1995), it is possible that this species has disappeared due to habitat loss.
7. While SPI offers suitable habitats for *Aetheorhiza bulbosa* (L.) Cass., when not in flower it can easily be confused with the common *Scorpiurus muricatus* L., which has similar leaves and can grow in similar habitats; thus a misidentification cannot be ruled out.
8. *Atractyllis cancellata* L. still exists on the South part of the larger half, but from the comment "a good population" (Stevens, 2004), lack of sighting by J. Sciberras et al. (2012) and a very small population observed in our records, indicates that the population may be on the decline. However, since this is an annual, its populations are liable to fluctuate from year to year.
9. *Centaurea melitensis* L. was first confirmed by [EL] in early 1970's and Mario Gauci (in Haslam et al., 1977). The continuous lack of its sightings since, may suggest its extinction from the 'islet', but as it has been mentioned above, annuals tend to show population fluctuations from year to year.

10. *Hyoseris frutescens* Brullo & Pavone was first reported by J. Sciberras and Sciberras (2010), but was not observed in our surveys. Contact with one of the authors revealed that they saw a single young plant, which, from the photographs supplied, it must have been a recent introduction owing to the small size and lack of a woody stock. Establishment of this is therefore uncertain.

Table 1: List of plant species, including mosses and lichens recorded during four site visits (Oct-2014, Mar-2015, May-2015, Jun-2015) at Selmunett, including their approximate frequency on the islet (4=common; 3=frequent; 2=scarce; 1=rare, + = casual/very rare) and their current protection status. Species in bold type are first records from Selmunett.

Sp. Index Number	Taxa (Higher Plants)	Est. Frequency Oct-14	Mar-15	May-15	Jun-15	RDB	Protection by L.N.311	L.N.200
001	***Adiantum capillus-veneris***	+	+	+	+			
002	*Agave americana*	1	1	1	1			
003	*Agave sisalana*				+			
004	*Ajuga iva subsp. pseudo-iva*				1			
005	*Allium ampeloprasum complex*	3	2	3	2			
006	*Allium lojaconoi*				2	✓	✓	
007	*Aloe vera*	+	+	+	+	✓		
008	*Anacamptis pyramidalis*			2				
009	*Anagallis arvensis*		2	+				
010	*Anthemis urvilleana*	2	2	2				
011	*Arisarum vulgare*	2	2					
012	*Arthrocnemum macrostachyum*	4	4	4	4			
013	*Asparagus aphyllus*	2	2	2	2			
014	*Asphodelus aestivus*		+					
015	*Asteriscus aquaticus*	1	1	2	1			
016	*Astragalus hamosus*		3	2				
017	*Astragalus sesameus*		1	1				
018	*Atractylis cancellata*	1		1		✓	✓	
019	*Avena barbata*		+	+				
020	*Beta maritima*		1	1				
021	*Bituminaria bituminosa*	2	2	1				
022	*Borago officinalis*		1	+				
023	*Bromus hordaceus*				+			
024	*Bromus madritensis*		4	3	1			
025	*Capparis orientalis*	3	3	3	3			
026	***Carlina gummifera***			1	1			
027	*Carlina involucrata*	2	2	2	2	✓		
028	*Catapodium marinum*		1	1				
029	*Centaurium pulchellum*	1		1				
030	*Ceratonia siliqua*	1	1	1	1	✓		✓
031	*Cerinthe major*		1					
032	*Chenopodiastrum murale*		+	+				
033	*Chiliadenus bocconei*	3	2	2	3			
034	*Convolvulus elegantissimus*				+			
035	*Convolvulus oleifolius*	2	2	2	2	✓	✓	
036	*Coronilla scorpioides*		+					
037	*Cuscuta epithymum*		+					
038	*Cynara cardunculus*	3	3	3	1			

039	*Dactylis glomerata* subsp. *hispanica*	2	1	2				
040	*Daucus carota*	3	2	2	2			
041	*Daucus gingidium*		3	3	3			
042	*Desmazeria pignattii*			+			✓	
043	*Dittrichia viscosa*	+	1	1	1			
044	*Ecballium elaterium*	+						
045	*Echium arenarium*			1				
046	*Echium parviflorum*		1	1				
047	*Erica multiflora*		+					
048	*Erodium cicutarium*		+	+				
049	*Erodium malacoides*		3	1				
050	*Euphorbia exigua* s.l.		2	2				
051	*Euphorbia peplus* subsp. *peploides*		+	+				
052	*Euphorbia pinea*	2	2	1	1			
053	*Ferula communis*	3	3	2	1			
054	*Filago pygmeus*		1	+			✓	
055	*Frankenia hirsuta*	2	2	2	1			
056	*Galactites elegans*		2	1				
057	***Galium murale***		+					
058	***Gladiolus* sp.**		1					
059	*Hedypnois rhagadioloides*		1	+				
060	*Hedysarum coronarium*		1	1				
061	***Heliotropium europaeum***				1			
062	***Hippocrepis biflora***		1					
063	*Hyoseris scabra*		2					
064	***Hyparrhenia hirta***	1			1			
065	*Hypericum triquetrifolium*				2			
066	*Iris pseudopumila*		+			✓		
067	*Jacobaea maritima subsp. sicula*	3	3	3	3			
068	*Lagurus ovatus*	+	1		+			
069	*Limbarda crithmoides*	4	4	4	4			
070	*Limonium melitense*	3	3	3	3	✓	✓	
071	*Limonium virgatum*			2	2	✓		
072	*Limonium zeraphae*			+	2	✓	✓	
073	*Linaria pseudolaxiflora* (Fig. 1C)		+			✓	✓	
074	*Linum strictum*			+				
075	*Lobularia maritima*	1	2	1	1			
076	*Rostraria cristata*			+				
077	*Lotus cytisoides*	2	2	2	2			
078	*Lotus edulis*		4	2				
079	*Lotus ornithopodioides*		2	+				
080	***Lotus tetragonolobus***		2					
081	*Lygeum spartum*	1			1			
082	*Malva parviflora*		2	1	1			
083	*Medicago littoralis*		1	1				
084	***Medicago lupulina***		1	+				
085	*Medicago monspeliaca*		1					
086	*Medicago polymorpha*		1					
087	*Melilotus indicus*		2	1				

088	*Mercurialis annua*		1					
089	*Mesembryanthemum nodiflorum*	2	2	2	1			
090	*Micromeria microphylla*	1	1		1	✓		
091	*Muscari comosum*			+				
092	*Narcissus tazetta*		3	1				
093	***Ophrys bombyliflora***		+					
094	*Opuntia ficus-indica*	+	+					
095	*Opuntia stricta var. stricta*	3	3	3	3			
096	***Ornithogalum narbonense***			1				
097	***Orobanche cernua*** (Fig. 1B)			1		✓		
098	***Orobanche* cf. *minor***			1				
099	***Orobanche pubescens***			1				
100	*Oxalis pes-caprae*	1	1					
101	*Pallenis spinosa*			+				
102	*Parapholis incurva*		+	1				
103	*Parietaria cretica* (Fig. 1A)		1	1				
104	*Parietaria judaica*	1	1	1	1			
105	*Periploca angustifolia*	1	1	1	1	✓		
106	*Phagnalon rupestre* subsp. *graecum* var. *ginzbergeri*	2	2	2	2			
107	***Pistacia lentiscus***	+	+	+	+			✓
108	*Plantago coronopus* s.l.		3	3				
109	*Plantago lagopus*			+				
110	***Plocama (=Putoria) calabrica*** (Fig. 1D)	+	+	+	+	✓	✓	
111	*Polypogon maritimus*			+				
112	*Prasium majus*			+	+			
113	***Prospero autumnalis***	3						
114	*Reichardia picroides*	+	1					
115	*Romulea cf. ramiflora* s.l.		2	1				
116	*Rubia peregrina*	1		1				
117	*Sagina maritima*			+				
118	*Scorpiurus muricatus*		2	1				
119	*Sedum caeruleum*		2	1				
120	***Sedum litoreum*** (Fig. 1E)			1		✓		
121	*Sedum rubens*		2	2				
122	*Sedum sediforme*	+	+	+	+			
123	*Sideritis romana*	2	2	2	1			
124	*Silene sedoides*		2	3	1			
125	*Silene vulgaris*			+				
126	*Sonchus oleraceus*		1	1	1			
127	*Sonchus tenerrimus*		1	1	1			
128	***Spergularia diandra***			1				
129	*Suaeda vera*			1	1			
130	*Teucrium fruticans*	1	1	1	1			
131	*Theligonum cynocrambe*		1					
132	*Trachynia distachya*							
133	*Trifolium scabrum*		+	+				

134	*Trifolium stellatum*		+	+				
135	*Umbilicus horizontalis*	1	1	1	1			
136	*Urginea pancration*	2	2	2		✓		
137	*Urospermum picroides*		+					
138	*Valantia muralis*	2	2	2	1			
139	*Cymodocea nodosa*	2	2	2	2		✓	
140	*Posidonia oceanica*	2	2	2	2		✓	

Sp. Index Number	Taxa (Lichens)	Est. Frequency Oct-14	Mar-15	May-15	Jun-15
501	***Caloplaca alociza***			3	
502	***Caloplaca aurantia***			4	
503	***Caloplaca chalybeia***			3	
504	***Caloplaca erythrocarpa***			3	
505	***Caloplaca flavescens***			4	
506	***Caloplaca inconnexa***			3	
507	***Caloplaca lactea* var. *lactea***			2	
508	***Caloplaca marmorata***			3	
509	***Caloplaca subochracea***			3	
510	***Catillaria detractula***			2	
511	***Clauzadea metzleri***			3	
512	*Collema tenax*			3	
513	***Diploicia canescens***			2	
514	***Dirina ceratoniae***			2	
515	***Dirina massiliensis*** (Fig. 2A)			3	
516	***Lecania spadicea***			4	
517	***Lecanora lisbonensis*** (Fig. 2C)			3	
518	***Opegrapha calcarea***			4	
519	***Opegrapha rupestris***			3	
520	***Placidium tenellum***			3	
521	***Protoparmeliopsis muralis*** (Fig. 2B)			2	
522	*Psora decipiens*			3	
523	*Roccella phycopsis* (Fig. 2D)			2	
524	***Verrucaria calciseda***			4	
525	***Verrucaria nigrescens***			4	
526	***Xanthoria calcicola***			3	
527	*Xanthoria parietina*			2	

Sp. Index Number	Taxa (Mosses)	Est. Frequency Oct-14	Mar-15	May-15	Jun-15
601	***Entosthodon pulchellus***			1	
602	*Tortella flavovirens*			2	
603	***Trichostomum brachydontium***			2	

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Table 2: List of plants recorded from Selmunett from different visits including historical records and compared to our 143 records (mauve columns). Legend used in this table is * = first record from Selmunett, ! = observed/recorded, ? = doubtful identity, x = records from previous excursions but not observed in our four visits. Further taxonomic or relevant information is given to taxa followed by a numerical superscript, where the number corresponds to the note given in the notes section further below. Species present in the smaller part of the islet are also indicated in the last row of the table. Number in square brackets correspond to additional notes discussed further below.

Family	Species	Borg 1927	EL 1973	EL 1983	PJS/EL 1983	EL 1990	EL 1995	FR 1995	DTS+ 2000	DTS+ 2004	JS+AS 2012	EL+SM Oct-14	EL+SM Mar-15	All May-15	EL+SM Jun-15	**Comb. 2014–2015**	Sp. No.	Smaller islet
Aizoaceae	Mesembryanthemum nodiflorum [1]	*		!				!	!	!	!	!	!	!	!	**!**	1	✓
Amaranthaceae	*Arthrocnemum macrostachyum*		*	!	!			!	!	!	!	!	!	!	!	**!**	2	✓
	Beta maritima						*				!		!	!		**!**	3	
	Chenopodium murale		*	!									!	!		!	4	
	Darniella melitensis [2]					*				!	!					x	5	
	Halimione portulacoides [3]										*					x	6	
	Suaeda vera		*	!					!	!				!	!	!	7	✓
Amaryllidaceae	*Allium ampeloprasum* [4]	*		!								!	!	!	!	!	8	
	Allium commutatum [4]						*		!							x	9	
	Allium lojaconoi		*	!											!	!	10	
	Allium melitense [4]					*			!	!						-	11	
	Narcissus tazetta		*	!	!			!	!	!	!		!	!		!	12	
	Pancratium maritimum [3]										*					x	13	
Anacardiaceae	*Pistacia lentiscus*											*	!	!	!	!	14	
Apiaceae	*Daucus carota* [5]					*			!	!		!	!	!	!	!	15	
	Daucus (cf.) *rupestris* [5]		*	!	!				!		!					!	16	
	Daucus gingidium [5]									*	!		!	!	!	!	17	✓
	Crithmum maritimum										*					x	18	
	Ferula communis		*	!	!			!	!	!		!	!	!	!	!	19	✓
	Ridolfia segetum [6]	*														x	20	
	Tordylium apulum		*	!												x	21	
Araceae	*Arisarum vulgare*								*		!	!	!			!	22	✓
	Arum italicum								*		!					x	23	
Areaceae	*Chamaerops humilis*										*					x	24	
Asclepiadaceae	*Periploca angustifolia*								*	!	!	!	!	!	!	!	25	
Asparagaceae	*Agave americana*						*		!	!	!	!	!	!	!	!	26	
	Agave sisalana									*	!				!	!	27	
	Asparagus aphyllus		*	!					!	!	!	!	!	!	!	!	28	
	Muscari comosum										*			!		!	29	
	Ornithogalum arabicum		*	!							!					x	30	
	Ornithogalum narbonense													*		!	31	
	Prospero autumnalis											*				!	32	
	Scilla sicula										*					x	33	
	Urginea pancration	*		!					!	!	!	!	!	!		!	34	✓
Asteraceae	Aetheorhiza bulbosa [7]								*							x	35	
	Anthemis urvilleana	*		!					!	!		!	!	!		!	36	✓
	Atractylis cancellata [8]					*			!	!		!		!		!	37	
	Atractylis (=Carlina) gummifera													*	!	!	38	
	Asteriscus aquaticus		*	!					!	!	!	!	!	!	!	!	39	
	Bellis annua		*	!							!					x	40	

	Carduus australis subsp. marmoratus					*										x	41	
	Carlina involucrata					*			!	!	!	!	!	!	!	!	42	
	Centaurea melitensis [9]			!												x	43	
	Jasonia (=Chiliadenus) bocconei	*		!					!	!	!	!	!	!		!	44	
	Cynara cardunculus		*	!				!	!	!	!	!	!	!	!	!	45	
	Dittrichia graveolens	*														x	46	
	Dittrichia viscosa		*	!					!	!	!	!	!	!	!	!	47	
	Evax (=Filago) pygmaea	*							!	!	!		!	!		!	48	
	Galactites tomentosa (= G. elegans)		*	!					!	!	!		!	!		!	49	
	Hedypnois rhagadioloides		*	!					!	!			!	!		!	50	
	Hyoseris frutescens [10]										*					x	51	
	Hyoseris radiata								*							x	52	
	Hyoseris scabra								*				!			!	53	
	Inula (=Limbarda) crithmoides		*	!	!			!	!	!	!	!	!	!	!	!	54	✓
	Pallenis spinosa	*												!		!	55	
	Phagnalon graecum subsp. *ginzbergeri*								*	!	!	!	!	!	!	!	56	
	Reichardia picroides		*	!					!	!	!	!	!			!	57	
	Senecio bicolor (=Jacobaea maritima subsp. *sicula)*	*		!					!	!	!	!	!	!	!	!	58	
	Senecio pygmaeus								!							x	59	
	Sonchus oleraceus								!				!	!		!	60	✓
	Sonchus tenerrimus		*	!					!	!	!		!	!	!	!	61	✓
	Urospermum picroides								!				!			!	62	
Boraginaceae	*Borago officinalis*		*	!					!	!	!		!	!		!	63	
	Cerinthe major					*							!			!	64	
	Echium arenarium	*				!			!	!	!			!		!	65	✓
	Echium parviflorum	*				!			!	!			!	!		!	66	
	Heliotropium europaeum														*	!	67	
Brassicaceae	*Matthiola incana subsp. melitensis*										*					x	68	
	Lobularia maritima		*	!					!	!	!	!	!	!	!	!	69	
Cactaceae	*Opuntia ficus-indica*				*			!	!			!	!			!	70	
	Opuntia stricta var. *stricta*					*			!	!	!	!	!	!	!	!	71	
Capparidaceae	*Capparis orientalis*		*	!	!			!	!	!	!	!	!	!	!	!	72	✓
Caryophyllaceae	*Sagina apetala* [11]		*	!												x	73	
	Sagina maritima [11]													*		!	74	
	Silene sedoides		*	!					!	!	!		!	!	!	!	75	✓
	Silene vulgaris								*					!		!	76	
	Spergularia diandra [12]													*		!	77	
	Spergularia bocconei [12]		*	!						!						x	78	
	Spergularia marina		*	!					!							x	79	
Convolvulaceae	*Convolvulus elegantissimus*					*				!					!	!	80	
	Convolvulus oleifolius										*	!	!	!	!	!	81	
	Cuscuta epithymum								*				!			!	82	
Crassulaceae	*Sedum caeruleum*		*	!					!	!	!		!	!		!	83	
	Sedum litoreum [13]													*		!	84	✓

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	Sedum rubens		*		!					!		!				!		!	85	
	Sedum sediforme												*		!	!		!	86	
	Umbilicus horizontalis		*		!					!			!		!	!		!	87	
Cucurbitaceae	*Ecballium elaterium*		*		!										!			!	88	
Dipsaceae	*Sixalix atropurpurea* subsp. *maritima* [14]									*								x	89	
Ericaceae	*Erica multiflora*									*						!		!	90	
Euphorbiaceae	*Euphorbia exigua* s.l. [15]		*		!					!			!			!		!	91	
	Euphorbia peplus subsp. *peploides*		*		!					!						!		!	92	
	Euphorbia pinea	*			!	!			!	!	!			!		!		!	93	✓
	Mercurialis annua		*		!					!						!		!	94	
Fabaceae	*Anthyllis vulneraria*												*					x	95	
	Astragalus hamosus		*		!					!		!	!			!		!	96	
	Astragalus sesameus						*									!		!	97	
	Bituminaria bituminosa		*		!					!					!	!		!	98	
	Ceratonia siliqua [16]									*		!	!		!	!		!	99	
	Coronilla scorpioides						*			!		!				!		!	100	
	Hedysarum coronarium		*		!				!	!		!				!		!	101	
	Hippocrepis biflora															*		!	102	
	Lotus cytisoides						*			!		!	!		!	!		!	103	
	Lotus edulis		*		!					!		!	!			!		!	104	
	Lotus ornithopodioides									*		!				!		!	105	
	Lotus tetragonolobus															*		!	106	
	Medicago littoralis						*			!		!				!		!	107	
	Medicago monspeliaca						*			!		!				!		!	108	
	Medicago polymorpha									*						!		!	109	
	Medicago lupulina															*		!	110	
	Melilotus indicus						*			!		!				!		!	111	✓
	Melilotus sulcatus									*								x	112	
	Ononis mitissima		*		!													x	113	
	Scorpiurus muricatus		*		!					!		!				!		!	114	
	Trifolium scabrum						*			!		!	!			!		!	115	
	Trifolium stellatum	*								!		!				!		!	116	
	Trifolium tomentosum [17]						*											x	117	
Frankeniaceae	*Frankenia hirsuta*		*		!					!		!	!		!	!		!	118	✓
	Frankenia pulverulenta		*		!								!					x	119	
Gentianaceae	*Blackstonia perfoliata*		*		!													x	120	
	Centaurium pulchellum		*		!					!		!				!		!	121	
Geraniaceae	*Erodium cicutarium*									!						!		!	122	
	Erodium malacoides		*		!					!		!				!		!	123	✓
	Geranium molle [18]									*								x	124	
	Geranium rotundifolium 18]												*					x	125	
Hypericaceae	*Hypericum triquetrifolium*		*		!							!	!				!	!	126	
	Hypericum cf. *australe* [19]						*											x	127	
Iridaceae	*Gladiolus* sp.															*		!	128	
	Iris pseudopumila [20]												*			!		!	129	
	Iris sicula												*					x	130	
	Romulea columnae [21]	*			!								!					x	131	
	Romulea ramiflora [21]									*		!				!		!	132	
	Romulea rollii [21]									!								x	133	✓
Lamiaceae	*Ajuga iva* s.l. [22]									!		!						x	134	

	Ajuga iva subsp. *pseudoiva* [21]		*	!											!	!	135		
	Micromeria microphylla				*			!	!	!	!	!			!	!	136		
	Prasium majus						*			!			!			!	137		
	Sideritis romana [23]		*	!				!	!	!	!	!	!	!	!	!	138		
	Teucrium fruticans		*	!				!	!		!	!	!	!	!	!	139		
	Thymus capitatus	*														x	140		
Linaceae	*Linum strictum*				*			!	!	!			!			!	141		
Malvaceae	*Malva parviflora*		*	!				!	!	!		!	!	!		!	142		
Moraceae	*Ficus carica* var. *caprificus*		*	!				!		!						x	143		
Oleaceae	*Olea europaea*									*						x	144		
Orchidaceae	*Anacamptis pyramidalis*		*	!						!			!			!	145		
	Anacamptis urvilleana									*						x	146		
	Ophrys bombyliflora											*				!	147		
	Orchis collina									*						x	148		
	Orchis coriophora subsp. *fragrans*		*	!				!								x	149		
Orobanchaceae	*Orobanche mutelii*		*	!				!	!							x	150		
	Orobanche cernua												*			!	151		
	Orobanche cf. *minor*												*			!	152		
	Orobanche pubescens												*			!	153		
Oxalidaceae	*Oxalis pes-caprae*		*	!			!	!		!	!	!				!	154		
Papaveraceae	*Fumaria officinalis*									*						x	155		
Plantaginaceae	*Linaria pseudolaxiflora* [24]				*							!				!	156		
	Plantago commutata [25]							!	!							x	157		
	Plantago coronopus [25]	*		!						!	!	!	!			!	158		
	Plantago crypsoides [25]				*			!	!							-	159		
	Plantago lagopus	*								!			!			!	160		
	Plantago weldenii [25]							!								x	161		
Plumbaginaceae	*Limonium melitense* [26]							!	!	!	!	!	!	!		!	162	✓	
	Limonium virgatum [26]	*							!	!			!	!		!	163		
	Limonium zeraphae [26]	*		!				!					!	!		!	164	✓	
Poaceae	*Avena barbata*				*			!				!	!	!		!	165	✓	✓
	Avena hirtula								*							x	166	✓	
	Avena ludoviciana				*				!							x	167		
	Bromus fasciculatus				*			!	!							x	168		
	Bromus hordaceus				*			!						!		!	169		
	Bromus madritensis		*	!				!	!	!		!	!			!	170		
	Bromus rigidus							*								x	171	✓	
	Catapodium marinum		*	!				!				!	!			!	172	✓	✓
	Catapodium rigidum		*	!				!								x	173	✓	
	Cutandia maritima [27]		*	!												x	174		
	Dactylis glomerata subsp. *hispanica*				*			!	!	!	!	!	!			!	175		
	Desmazeria pignattii							!	!				!			!	176	✓	
	Hyparrhenia hirta										*	!		!		!	177		
	Lagurus ovatus				*			!	!	!	!	!				!	178		
	Lygeum spartum							*	!		!			!		!	179		
	Lophochloa cristata		*	!				!	!				!			!	180		
	Parapholis filiformis [18]									*						x	181		

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	Parapholis incurva [18]		*	!					!	!			!	!		!	182	✓
	Polypogon maritimus													!		!	183	
	Polypogon subspathaceus		*	!												x	184	
	Stipa capensis					*			!	!						x	185	
	Trachynia distachya					*			!	!			!	!		!	186	
Primulaceae	*Anagallis arvensis*		*	!					!	!	!		!	!		!	187	
Pteridaceae	*Adiantum capillus-veneris*											*	!	!	!	!	188	
Resedaceae	*Reseda lutea* [28]	*														x	189	
Rubiaceae	*Galium muralis*												*			!	190	
	Putoria (=Plocama) calabrica											*	!	!	!	!	191	
	Rubia peregrina						*			!		!		!		!	192	
	Sherardia arvensis	*														x	193	
	Theligonum cynocrambe		*	!					!				!			!	194	
	Valantia muralis	*		!					!	!	!	!	!	!	!	!	195	
Rutaceae	*Ruta chalepensis*										*					x	196	
Solanaceae	*Hyoscyamus albus*		*	!							!					x	197	
Urticaceae	*Parietaria cretica* [29]		*	!	!								!	!		!	198	
	Parietaria judaica										*	!	!	!	!	!	199	
Xantorrhoeaceae	*Aloe vera*				*			!				!	!	!	!	!	200	
	Asphodelus aestivus		*	!					!		!	!	!			!	201	
Cymodoceaceae	*Cymodocea nodosa*		*	!								!	!	!	!	!	202	✓
Posidoniaceae	*Posidonia oceanica*		*	!					!	!		!	!	!	!	!	203	✓
Totals	203	23	68	80	10	32	4	12	115	83	89	55	107	107	55	143		31

11. *Sagina apetala* Ard./*Sagina maritima* G.Don; Both species are very closely related and easy to confuse with each other. Selmunett offers the habitat for both, but our latest observations confirmed only the presence of *S. maritima* putting doubt in the previous records of *S. apetala*.
12. *Spergularia diandra* (Guss.) Heldr. is recorded for the first time, but previous records of *S. bocconei* might also be referable to this species, though it is possible that both species exist since both are frequent in the same type of habitat and are often difficult to tell apart in the field.
13. A few small clumps of *Sedum litoreum* Guss. (Fig. 1E), each with numerous specimens, were found only on the smaller part of the 'islet'.
14. *Scabiosa atropurpurea* L. (Stevens, 2000) was a tentative identification on a plant found in leaves. Later it was mooted to be instead *S. romana* L. (Stevens, 2004). Given that no *Scabiosa* spp. was recorded, we concur to reject this species from the florula of Selmunett.
15. *Euphorbia exigua* is here treated in a wide sense. Most of the plants we have seen are referable to the var. *pycnophylla* K.U. Kramer & Westra, although some specimens intermediate between this and the var. *exigua* were also encountered. The validity of the var. *pycnophylla*, originally described from Malta and subsequently also found on the island of Lampedusa, needs to be established since it may only be an ecological variant.
16. According to Stevens (2004) the carob trees (*Ceratonia siliqua* L.) are introduced due to their *"unnatural distribution"*. We concur with this observation, also because the islet does not offer any true maquis habitat and it was common practice for farmers to plant this tree for fodder and shade. On the other hand, since the islet was once cultivated agriculturally, it is reasonable to suppose that the farmer(s) would also have planted carobs, especially close to the farmhouse and around the agricultural land; hence relicts might have survived.
17. *Trifolium tomentosum* L. used to be frequent in the Maltese Islands, but it has now become quite rare and thus might have disappeared from the islet as part of its general rapid decline.
18. We are confident that *Geranium rotundifolium* L. was misidentified with the closely related *G. molle*, and similarly *Parapholis incurva* (L.) C.E.Hubb. with *P. filiformis* (Roth) C.E.Hubb. since Selmunett does not offer the habitat for either species.
19. A single sighting of what appeared to be a *Hypericum* species (Lanfranco, 1990) was doubtfully identified as *H. australe* Ten. Its identity could not be confirmed since it was not yet in flower during that visit. Moreover, since only one small specimen was seen, no material was collected for examination in the lab. It has not been observed again during subsequent visits and, considering that the habitat of this species (humid woodland) is not present at Selmunett, we now suggest to remove this doubtful species from the *florula* of the islet.
20. Two plants of *Iris pseudopumila* Tineo were found in the middle of an abandoned field dominated by recent formations of degraded steppic vegetation based on thistles and agrospecies. Considering this native species has a low seed production, and the very small population (2 plants), suggests that this is a very recent introduction.
21. All specimens of *Romulea* spp. observed during the second visit corresponded to the new taxon *R. variicolor* S. Mifsud s.l., following a recent revision of the genus for the Maltese islands (S. Mifsud, 2015). The additional presence of *R. columnae*, also recorded in some previous surveys, is not excluded since the islet does offer its habitat. On the other hand, *Romulea rollii*, which typically occurs in sand dunes, does not occur on the Maltese islands (S. Mifsud, 2015) and should similarly be removed from the florula of Selmunett.
22. All 10–12 specimens of *Ajuga iva* (L.) Schreb. observed during the fourth visit had yellow flowers, and hence correspond to *Ajuga iva* subsp. *pseudo*-iva (Labill. & Castagne ex DC.) Holmboe, thus concurring with observations made by Lanfranco (1973, 1983).
23. Some specimens of *Sideritis romana* L. were very large when compared to typical plants as they normally grow in Malta. These had long and foliose ascending stems reaching over 30 cm.
24. Only two plants of *Linaria pseudolaxiflora* Lojac. (Fig. 1C) in the same locus were observed. This annual species may be facing local extinction from Selmunett, although, as noted above, annuals tend to fluctuate from year to year.
25. *Plantago commutata* Guss, *P. crypsoides* Boiss., *P. bombycina* Sommier & Caruana Gatto and *P. weldenii* Rchb. are closely related taxa within the *Plantago coronopus* L. aggregate, and all of which, have been reported from Malta. Due to the complexity of this aggregate, we are provisionally treating these taxa as *P. coronopus* s.l.
26. In our surveys *Limonium zeraphae* Brullo, *L. melitense* Brullo and *L. virgatum* (Willd.) Fourr. have been confirmed together for the first time. Some specimens with intermediate characters, especially between *L. virgatum* and *L. melitense* have been observed concurring with previous ob-

servations.

27. The old record of *Cutandia maritima* (L.) Benth. (Lanfranco, 1973), was based on a tiny specimen with just two or three reduced spikelets which, at the time, seemed to be to some extent comparable with *Cutandia maritima*. Since in the last 30 years, typical specimens were not reported again, we now believe that it might have been confused with a decrepit *Catapodium rigidum* (L.) C.E.Hubb. or *Catapodium marinum* (L.) C.E.Hubb., and at present, it safe to exclude *C. maritima* from the florula of Selmunett.
28. Lanfranco (1983) included *Reseda lutea* L. as a record by Borg (1927), but Stevens (2004), pointed out that this was not listed by Borg (1927) and should be rejected from the florula of Selmunett, which we concur.
29. *Parietaria cretica* L. was last seen in 2000 and had apparently disappeared from sight on subsequent excursions and surveys. Stevens (2004) commented: "Although thoroughly searched for in the area from where it was formerly recorded, this plant was not observed." J. Sciberras et al. (2012) did not observe it either and shared worries with Stevens (2004) and Lanfranco (2014) (pers. comm.) of its possible extinction since Selmunett is the only site in the Maltese islands where *P. cretica* is known to occur (Lanfranco, 1989). Similarly, in a statement by the Nature Trust on the occasion of World Biodiversity Day, the NGO said: "biodiversity officers had not seen any specimens of the endemic [sic] plant *Parietaria cretica* or the endemic subspecies of the Maltese wall lizard (*kieselbachi*) in site visits over the last two years." (Times of Malta, 2006, May 23). During this survey [SM] rediscovered scattered specimens (Fig. 1A) close to its original station on the 14th March 2015, as well as other small clumps to the south and the east of the larger 'islet'. Identity was confirmed by [EL] *in situ*. Hence, our study confirms that the species is still extant on Selmunett, and even has a wider distribution then what previous reports suggest, with at least three separate stations. As stated in the Red Data Book (Lanfranco, 1989), the status of this species is endangered and a restricted species for the Mediterranean and Maltese Islands.

Table 3: Plant species recorded from Selmunett prior our visit and not found in our surveys. The species' life cycle, its last record, and total number of times it has been recorded from Selmunett (1927 to 2012) is given. Species flagged with # are in our opinion past misidentifications; those with † are most likely casual or accidental records, while those with †† are presumably genuine cases of extinction from Selmunett - based on the fact that they are old records of perennial plants and mostly recorded once.

Family	Species	Life Cycle	Last record	Number of records
Amaranthaceae	*Salsola melitensis* #	Perennial	Lanfranco (1990)	3
	Halimione portulacoides †(#)	Perennial	J. Sciberras et al. (2012)	1
Amaryllidaceae	*Pancratium maritimum* †(#)	Perennial	J. Sciberras et al. (2012)	1
Apiaceae	*Crithmum maritimum* †	Perennial	J. Sciberras et al. (2012)	1
	Ridolfia segetum ††	Annual	Borg (1927)	1
	Tordylium apulum	Annual	Lanfranco (1973)	1
Araceae	*Arum italicum*	Perennial	Stevens (2000)	2
Areaceae	*Chamaerops humilis* †	Perennial	J. Sciberras et al. (2012)	1
Asparagaceae	*Allium commutatum*	Perennial	Lanfranco (1995)	2
	Ornithogalum arabicum ††	Perennial	Lanfranco (1973)	2
	Scilla sicula †	Perennial	J. Sciberras et al. (2012)	1
Asteraceae	*Aetheorhiza bulbosa* #	Perennial	Stevens (2000)	1
	Bellis annua	Annual	Lanfranco (1973)	2
	Carduus australis subsp. *marmoratus* †	Annual	Lanfranco (1990)	1
	Centaurea melitensis ††	Annual	Haslam et al. (1977)	1
	Dittrichia graveolens ††	Annual	Borg (1927)	1
	Hyoseris frutescens †	Perennial	J. Sciberras et al. (2012)	1
	Hyoseris radiata	Perennial	Stevens (2000)	1
	Senecio pygmaeus	Annual	Stevens (2000)	1

Brassicaceae	*Matthiola incana* subsp. *melitensis* †	Biennnial/Peren.	J. Sciberras et al. (2012)	1
Caryophyllaceae	*Sagina maritima*	Annual	Lanfranco (1973)	1
	Spergularia bocconei	Annual/Biennial	Lanfranco (1973)	2
	Spergularia marina	Annual	Lanfranco (1973)	2
Hypericaceae	*Hypericum cf. australe* ††(#)	Perennial	Lanfranco (1990)	1
Fabaceae	Anthyllis vulneraria †	Perennial	J. Sciberras et al. (2012)	1
	Melilotus sulcatus	Annual	Stevens (2000)	1
	Ononis mitissima	Annual	Stevens (2000)	1
	Trifolium tomentosum ††	Annual	Lanfranco (1990)	1
Frankeniaceae	*Frankenia pulverulenta*	Annual	Lanfranco (1973)	2
Gentianaceae	*Blackstonia perfoliata* ††	Annual	Lanfranco (1973)	1
Geraniaceae	*Geranium molle*	Annual	Stevens (2000)	1
	Geranium rotundifolium #	Annual	J. Sciberras et al. (2012)	1
Iridaceae	*Iris sicula* †	Perennial	J. Sciberras et al. (2012)	1
	Romulea columnae	Perennial	Borg (1927)	3
	Romulea rollii #	Perennial	Stevens (2000)	1
Lamiaceae	*Ajuga iva* s.l.	Perennial	Stevens (2000)	2
	Thymbra capitata †	Perennial	Borg (1927)	1
Moraceae	*Ficus carica var. caprificus*	Perennial	Lanfranco (1973)	3
Oleaceae	*Olea europaea* †	Perennial	J. Sciberras et al. (2012)	1
Orchidaceae	*Anacamptis urvilleana*	Perennial	J. Sciberras et al. (2012)	1
	Orchis coriophora subsp. *fragrans*	Perennial	Stevens (2000)	2
Orobanchaceae	*Orobanche mutelii*	Perennial	Lanfranco (1973)	3
Papaveraceae	*Fumaria officinalis* †	Annual	J. Sciberras et al. (2012)	1
Plantaginaceae	*Plantago commutata*	Annual/Peren.	Stevens (2000)	2
	Plantago weldenii	Annual/Peren.	Stevens (2000)	1
Poaceae	*Avena hirtula*	Annual	Stevens (2004)	1
	Avena ludoviciana	Annual	Lanfranco (1990)	2
	Bromus fasciculatus	Annual	Lanfranco (1990)	3
	Bromus rigidus	Annual	Stevens (2000)	1
	Catapodium rigidum	Annual	Lanfranco (1973)	2
	Parapholis filiformis #	Annual	J. Sciberras et al. (2012)	1
	Polypogon subspathaceus	Annual	Lanfranco (1973)	2
	Stipa capensis	Annual	Lanfranco (1990)	3
Resedaceae	*Reseda lutea* #	Biennial	Borg (1927)	1
Rubiaceae	*Sherardia arvensis* ††	Annual	Borg (1927)	1
Rutaceae	*Ruta chalepensis* †	Perennial	J. Sciberras et al. (2012)	1
Solanaceae	*Hyoscyamus albus* †	Annual/Biennial	Lanfranco (1973)	2

Species recorded once by Borg (1927)	5 (3 ann + 2 per)
Species recorded once by Lanfranco (1973) incl. *Centaurea melitensis* and by Gauci in Haslam et al. (1977)	4 (annuals)
Species recorded once by Lanfranco (1990)	3 (annuals)
Species recorded once by Stevens (2000)	9 (7 ann + 2 per)
Species recorded once by Stevens (2004)	1 (annual)
Species recorded once by J. Sciberras et al. (2012)	16 (5 ann + 11 per)

5 Discussion

One of us [EL] has been visiting Selmunett over several decades, starting in the early 1970s. At that time the islet was infested with rabbits. Eventually the rabbits died out, apparently due to a disease. Since the large rabbit population exerted considerable pressure on the vegetation, following their disappearance the number of species shot up. In addition, whereas many of the plants used to be smaller than the mainland counterparts, following the extinction of the rabbits, the plants started to grow normally. A case in point are the grasses. When there were still rabbits only a very few species were recorded and they were largely stunted but, following the rabbit extinction, many more species were found and the majority were of normal size.

On a different note, during our 25–30 hours on the islets, we have not sighted any single reptile; reference here being made especially to the Selmunett lizard *Podarcis filfolensis kieselbachi* (Bedriaga, 1876), while several rat burrows have been encountered, especially at the southern part of the main 'islet'. Rat poison boxes were all abandoned. A few people wandering on the garigue were observed in three of the visits.

6 Conclusion

These four visits spread over one year have yielded some important results, namely 23 first records of lichens, 2 mosses and 20 higher plants, of which five vascular plants are strictly protected and one of them (*Parietaria cretica* L.) is a species confined only to Selmunett in the Maltese islands, had not been seen for some 15 years and was being doubted to have gone extinct. These visits have also shed light upon few taxa of uncertain status and an overall current update of the florula of the islet in order to help in the conservations of this Natura 2000 site and its plant species of ecological sensitivity. Given this update, we would encourage a much better protection of Selmunett, namely by frequent monitoring and better control of rats.

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Commentary

Institute of Space Sciences and Astronomy - Modified Gravity Research

Jackson Levi Said[*1], **Gabriel Farrugia**[1], **Andrew Finch**[1] **and Mark Pace**[1]
[1]*Institute of Space Sciences and Astronomy, University of Malta, Msida, MSD 2080, Malta*

1 The Gravity Research Group

Gravity forms an integral part of our everyday lives, but we rarely reflect on its inner workings. Its attractive pull is an essential part of understanding how many of the phenomena around us take place. Isaac Newton was the first physicist to put together a global approach to understanding the effects of gravity. However, it wasn't until Einstein that we got a holistic snapshot of how gravity works on larger scales.

In his model of gravity, Einstein gives us a picture of gravitational fields shaping the intrinsic structure of spacetime, such that particles no longer move in straight lines, but along the so-called "straightest possible line". This is the source of John Archibald's famous quote "Spacetime grips mass, telling it how to move... Mass grips spacetime, telling it how to curve" (Wheeler, 1999). This reshaping mechanism revolutionized the foundations of gravitational physics. The model passed all solar-system scale tests and reduced numerically to Newtonian Physics for weaker fields, such as in galaxy systems and clusters thereof. It wasn't long until problems started to arise. Firstly, galactic dynamics suffered from large deficiencies in that not enough gravitational pull was being produced to create the observed star dynamics. Moreover, going to the cosmological scale, that is, the observable universe as a whole, a repulsive effect was measured. While gravity appears to only attract for us, the cosmos appears to be expanding and at an accelerating rate. To account for this, two new concepts were put together, one being dark matter, which would account for the galactic dynamics. In proportion of about one (observed matter) is to six (dark matter), this would account for the stronger gravitational field effects.

On the cosmological setting, so-called dark energy would express a negative pressure on the universe, forcing it to expand. This would not translate to much for local phenomena, but on the cosmological scale it would force space itself to expand. In this way, observations would show stars and celestial objects to be moving apart.

Dark matter and dark energy may exist, however it may also be the case that gravity needs some tweaking in its description. This is where modified and alternative theories of gravity come in. The idea here is to take Einstein's working model of gravity and to extend it, either by adding parts that take hold at different scales (such as the galactic and cosmological scales or the quantum scale), or by completely reformulating the approach with the aim of recovering the Einstein model in the solar system.

Our approach is the latter of the last two, we work on teleparallel gravity. In this model gravity is equivalently reformulated as being expressed through torsion rather than curvature. The advantage in this case is that the resultant theory has a straightforward generalization, in the sense that its governing equations remain tractable. Moreover, the model incorporates an important idea in gravitational physics, called the equivalence principle. The equivalence principle concept is associated with how local dynamics are shielded from gravitational effects. In particular, an experiment being conducted in a laboratory will not be effected by the global gravitational field in the sense that the same outcome out be observed in a rocket being accelerated at the same rate.

Teleparallel gravity is built on elementary fields that relate these local systems with global coordinate systems. In this way, the model takes on an organic relation to how gravity works. Our group is working on studying the cosmological history of the universe within this model. We also work on studying how star and galaxy dynamics are effects by this change in model.

Jackson Levi Said

**Correspondence to*: Jackson Levi Said (jackson.said@um.edu.mt)

2 Cosmology and Gravity: The dark side of the universe

Einstein's theory of General Relativity (GR) has been very successful in predicting various phenomena, most recently that of gravitational waves (Abbott, 2016). The gravitational field, described as a curvature of space-time, has been used to try to explain how the universe came to be. From the earliest of time till now, the universe has been expanding, something which was observed by various astronomers in the 20^{th} century. However, an important independent discovery by the Supernova Cosmology Project and the High-Z Supernova Search Team in 1988 found that the universe was not only expanding, but expanding an accelerating rate (Riess, 1998).

This discovery led to various questions about the current best known theory of gravitation, since all observational components of the universe could not explain such acceleration. A possible alternative was proposed, a fluid with the property of exhibiting negative pressure, strong enough to counteract the force of gravity and force the universe to expand at an accelerating rate. This fluid, called dark energy, was and still is hypothetical leaving the theory of gravitation in a questionable state. If such a fluid did not exist, this leaves us with no possible explanation of this phenomenon. However, GR does not have to be a perfect theory, but rather a step forward closer to reality. This is the reason why alternative and modified theories of gravity exist, as an another and possibly a better way to describe the force of gravity.

Recently, the theory of teleparallelism and torsion has been revived, a concept which was originally proposed by Moller and later by Einstein in the 1920s, but left to wither as Einstein failed to unify electromagnetism to gravitation. Work in the 1960s and 1970s by various researchers (Cho, Pellegrini and Plebaski to name a few) revived this theory and showed that there was a link between describing torsion and gravitation. In fact, it was possible to equally describe gravitation as a manifestation of curvature in GR, through torsion in what was originally called New Gravity (now known as Teleparallel gravity) (De Andrade, Guillen & Pereira, 2000; Cai, Capozziello, De Laurentis & Saridakis, 2016).

Although being equivalent, the foundations are different, allowing links between local and global systems (those which are or are not affected by gravitation) and describing gravity back as a force. However, equivalence between the theories implied the same problems. Hence, some modifications to the theory were considered.

Teleparallel gravity describes gravitation through a Lagrangian, composed of a quantity which quantifies by how much the space-time has been twisted due to gravity called the torsion scalar, T. However, it might be the case that gravity does not simply function under this single scalar. Instead, this might be a part of a series of terms which fully describe its function. Therefore, one possible modification is by allowing some general function of the torsion scalar to take place in the Lagrangian, leading to what is called $f(T)$ gravity.

This modification results in changing the description of how the force of gravity works. Changing the Lagrangian results in a change in the equations of motion of the system. By doing so allows us to consider alternative ways to explain the aspect of dark energy without dark energy, completely through a manifestation of torsion of space-time and hence as a by-product of how gravity works.

Various models on this theory of gravity have been proposed (and are still being studied) as means to explain the expansion history of the universe; some of the most prominent candidates include power-law $f(T) = \alpha T^n$ by Bengochea and Ferraro (2009), exponential $f(T) = \alpha T_0 \left[1 - \exp\left(-p\sqrt{\frac{T}{T_0}}\right)\right]$ by Linder (2010) and logarithmic $f(T) = \alpha T_0 \sqrt{\frac{T}{qT_0}} \ln\left(\frac{qT_0}{T}\right)$ by Bamba, Geng, Lee and Luo (2011) (other competing models can be found in Nesseris, Basilakos, Saridakis & Perivolaropoulos, 2013), where α, p and q are model parameters, whilst T_0 is the torsion scalar evaluated at current times.

Although such models do prove to be successful in describing an expanding dark energy filled universe, this does not imply that everything is solved. Some models are good in describing specific phenomena, whilst others are good in describing others. A good model is one that is able to describe the various phenomena, whilst retaining the same Lagrangian. Therefore, it must be able to describe the various other problems found in the universe.

One such problem lies in the earliest stages of the universe, the Cosmic Microwave Background (CMB) radiation, discovered by Penzias and Wilson (1965). This radiation originates from the earliest moments of the universe's lifetime, where the universe was still extremely hot with photons colliding into each other continuously. As the universe expanded, it cooled down, leading to the formation of heavier elements. This resulted in a decrease in the number of collisions, allowing some photons to freely roam the space. This moment, called photon decoupling, leaves us an image of how the universe looked like when it was only 380,000 years old. It is this process that resulted in the CMB imprint today.

The first images of the CMB temperature distribution seemed to indicate that it was homogeneous and isotropic in every direction. However, advancements in technology allowed us to study temperature variations

in the scale of μK. Thanks to WMAP, and more recently Planck, the temperature distribution was in fact found to be anisotropic. Furthermore, it was found that the suggested expansion history of the universe did not agree with the size of the CMB spectrum, as the size of the universe at the CMB imprint suggests it is much larger.

How is this possible? Is there another unexplained mechanism causing this discrepancy? Various theories were proposed, however the most accepted one so far is that the universe undergoes an inflationary period, a stage where the size of the universe expanded e^{60} orders of magnitude. The true source which caused inflation is unknown (theory suggests a scalar field known as the inflaton field with an associated particle called the inflaton), however it is not attributed to standard matter and radiation, and is one which exhibits negative pressure in the same way as dark energy (Baaquie & Willeboordse, 2015; Dodelson, 2003). Whether a such field and particle truly exist is still unknown, the other possibility is that it can simply be a manifestation of gravity at such an early stages, which has not been explained so far. Therefore, the role of teleparallel gravity can be important as a possible alternative to explain this epoch.

Gabriel Farrugia

3 Galactic Rotation Dynamics in Modified Gravity

In the last hundred years, general relativity has proven to be an invaluable theory for explaining many properties of the universe. That being said, just as Newton's theory of gravity has its limits, so does general relativity. There are some areas where general relativity alone does not agree with observational data. Such an area is that of galactic rotation curves. Some of the most abundant formations in the observable universe are galaxies. Galaxies are accumulations of billions of stars, gasses and dust held together by gravity. When directly observing the multitude of galaxies surrounding our own, we notice that for general relativity to explain their behaviour, these galaxies would need far more mass then what can currently be observed.

Masses tend to orbit the center of their galaxy. Both theory and observation confirm that the orbital velocities of these masses tends to increase with radial distance from the galaxy's center until some point along the line a maximum orbital velocity is reached. It is here that a problem crops up. General relativity predicts that after this maximum velocity, these orbital velocities should start to diminish in magnitude until eventually going to zero. This dissipation of velocities is in conflict with what we actually observe when examining the behaviour of galaxies. According to our observational data (Chemin, Renaud & Soubiran, 2015), on various galaxies the orbital velocity of masses generally tends to plateau soon after reaching this maximum orbital velocity.

This phenomenon can be explained in General relativity by the introduction of non-luminous matter, dark matter. In order for dark matter to produce the required effect, it has to vastly outweigh the contribution of the luminous matter in a galaxy by a factor of roughly six. Although the addition of such a field is very successful in producing the correct rotation curves, dark matter itself has never been successfully directly observed. This discrepancy between theory and observation could also be an expression of the failure of general relativity to correctly describe the dynamics of the system in question. If this is so, our standard picture of gravity must change, thus making it necessary to construct alternative theories of gravity in the hopes of accounting for such a discrepancy (Mannheim & O'Brien, 2013).

One such alternative theory is that of torsional gravity. Apart from not treating gravity as a force, gravity in general relativity is curvature dominated. In torsional gravity, we treat gravity as a force manifesting from the torsion of the spacetime fabric. Since torsional gravity on its own reproduces the results of general relativity exactly, modifications of the theory are considered. Such theories are called $f(T)$ gravity theories. Here $f(T)$ represents functions dependant on T which is called the torsion scalar. Since galaxies consist of different parts with different geometric profiles, multiple expressions for the velocity profiles must be formulated in order to cater for these profiles. Various possible $f(T)$ functions will finally be tested in order to determine which can be solved and which are good candidates for further study.

The models constructed will be developed with the intention that they incorporate the observed velocity profiles of galactic rotation curves while still agreeing with other observational data with which general relativity is in agreement. It is only in the galactic regime where enough data exists to vigorously test these hypotheses, as well as have a strong enough field to allow for the appearance of deviations from the standard theory. It is for this reason that torsional gravity is being tested in this area. The work is being conducted in the hope of developing an $f(T)$ gravity theory to such a point that it can be employed in constraining its parameter set through observational data that is available freely.

Andrew Finch

4 Exotic Stars

Looking up at the night sky, you can't help but wonder at the vastness and endlessness of space. Countless stars dust the night sky in a symphony of light and spectacle. That is the romantic side to astrophysics. The amazing thing is that we have barely begun to scratch the surface on understanding what is really out there. Everyday new stars are being discovered. What we mostly work on are exotic types of stars. These are stars which were first theorized and later observed through sophisticated techniques.

Neutron stars are one of the most extreme objects in the Universe. They provide some of the best bases upon which we may test theories of gravity. They are like giant atom cores, kilometres in diameter, very dense, and violent. But how can something like this even exist? First, we shall take a look at the life of a star and how a neutron star is born.

The life of a star is a constant battle to keep two forces in balance - its own gravity and the radiation pressure propagated by its fusion reaction. In the core of a star, hydrogen atoms fuse together to form heavier elements. Heavier and heavier atomic nuclei fall and build up in the centre of the star. When the fusion reaction stops, the radiation pressure drops rapidly and the star would no longer be in balance. The outer layers of the star would be catapulted into space, in a violent explosion called *supernova*. The remnants of this explosion may form a neutron star depending on the residual core mass.

A neutron star's mass is nominally between $1\,\mathrm{M}_\odot$–$3\,\mathrm{M}_\odot$, but compressed to a celestial object about 25 km in diameter. A neutron star is very dense, so much so that one cubic centimetre of a neutron star contains the same mass as an iron cube 700 m, across roughly 10^9 tonnes. That is the same as having the mass of Mount Everest in the size of a sugar cube.

Since the density is so large, the gravity is bound to be very impressive, if one were to drop an object 1 m over the surface, it would hit the star in 1 µs and the object would reach a velocity of up to $2 \times 10^6\,\mathrm{m\,s^{-1}}$. The surface is very close to being perfectly flat, with irregularities of ±5 mm maximum. The surface temperature of a neutron star is about 10^6 K, as compared to 5800 K for our Sun.

The closer we get to the core, the more neutrons and fewer protons we see, until there's just an incredibly dense soup of indistinguishable neutrons. The cores of neutron stars are very unusual. We are still not sure what their properties are, but our closest guess is a *super-fluid neutron degenerate matter* or some kind of *ultra-dense quark matter, called quark-gluon-plasma*. This may only exist in such an ultra-extreme environment. In many ways, a neutron star is similar to a giant atom core. The difference is that while atom cores are held together by strong interactions, neutron stars are held together by gravity.

A few other extreme properties include the fact that neutron stars spin very fast. A younger neutron star would spin faster, and if there is a regular star nearby to feed it, it can rotate the neutron star up to several hundreds times per second.

An example is the neutron star PSR J1748-2246ad spins at approximately $252\,\mathrm{km\,h^{-1}}$ or 716 Hz. So fast that the star has a rather strange shape. We call these objects *pulsars* because they emit a strong radio signal. The magnetic field of a neutron star is roughly a trillion times stronger than the magnetic field of the Earth, so strong that atoms get bent when they enter its sphere of influence.

It is estimated that there are 10^8 neutron stars within our galaxy alone. Thus far, we have only observed about 1000 of these neutron stars ever since their discovery in 1960s.

Mark Pace

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Commentary

Neurosteroidogenesis: a New Pharmacological Target for Tourette Syndrome?

Roberto Frau*[1]
[1]*Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy*

Tourette Syndrome (TS) is a childhood-onset neurological disorder characterised by multiple motor tics and one or more phonic tics. Number, frequency and tic complexity vary over time. Although clinical and epidemiological studies indicate that TS is a partially inherited disorder, its pathogenesis remains poorly understood (Singer, 2005). One of the main features of TS is that its prevalence in children is significantly higher than in adults; in most TS patients, tic severity follows a characteristic time course, with onset at 5–7 years of age, followed by an increase in frequency and intensity until the age 10 and 12 years, and a later remission phase after puberty (Leckman et al., 1998).

Several clinical studies have highlighted male sex and environmental stress as risk factors in TS pathogenesis. TS has a strong male predominance, with a male:female ratio estimated at 4:1; furthermore, the severity of tics and accompanying symptoms (such as perceptual alterations, anxiety and obsessive-compulsive behaviours) are typically exacerbated by environmental stress (Cohen, Leckman & Bloch, 2013). In consideration of the role of steroids in the regulation of sex differences and stress response, these premises suggest that these molecules may be implicated in the etiology and pathophysiology of TS. In particular, our research focuses on neurosteroids, a family of steroids synthesized by the brain. A schematic diagram of steroidogenesis is presented in Fig. 1.

The key rate-limiting enzyme in androgens and neurosteroids synthesis, 5-alpha reductase (5AR), catalyses the saturation of the 4,5 double bond of the A ring of Δ4-3-ketosteroid substrates, such as deoxycorticosterone, progesterone, androstenedione and testosterone. Through this irreversible reaction, Δ4-3-ketosteroid substrates are converted into their pregnane and androstane

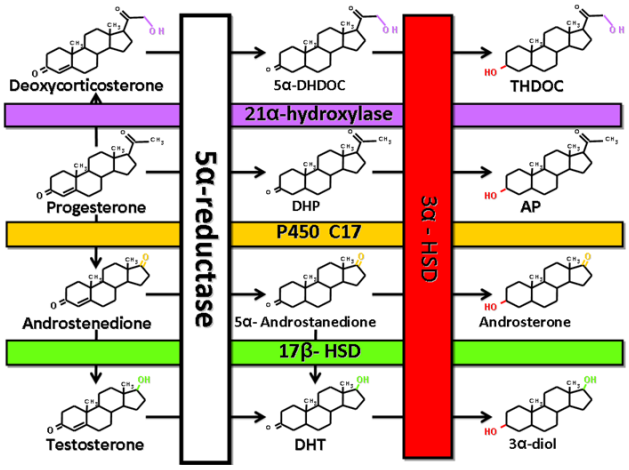

Figure 1: Simplified pathway of neurosteroidogenesis. 3-αHSD, 3-alpha hydroxysteroid dehydrogenase; P450-C17, steroid 17 alpha-hydroxylase/17,20 lyase; 17-β-HSD, 17-beta-hydroxysteroid dehydrogenase (modified from Paba et al., 2011).

metabolites. The physiological importance of 5AR in the brain derives from its capability to convert testosterone to the more potent brain active androgen dihydrotestosterone (DHT), and to convert progesterone and deoxycorticosterone to their respective 5alpha-reduced metabolites. These are precursors of allopregnanolone and tetrahydrodeoxycorticosterone, two neurosteroids directly implicated in the regulation of stress response through the positive modulation of the γ-amino-butyric acid (GABA)A receptor and HPA axis in response to stress. Among the five types of 5AR enzymes characterised to date, the first two isoforms (5AR1 and 5AR2) play major roles in brain steroidogenesis. The two isoforms are differently expressed and regulated: 5AR1 is the only isoform present in the glial

**Correspondence to*: Roberto Frau (roberto.frau@unica.it)

cells; the expression of 5AR2 is under the positive control of testosterone and DHT, while the expression of 5AR1 is negatively regulated by the same androgens.

Over the years, our group has demonstrated that the pharmacological inhibition of 5AR elicits therapeutic effects in animal models of TS (Bortolato et al., 2013). In particular, we found that the 5AR inhibitors finasteride and dutasteride normalised the disruption of sensorimotor gating, induced by the non-selective DAergic agonists amphetamine and apomorphine (Bortolato et al., 2008). Gating deficits, as measured by the paradigm of prepulse inhibition (PPI) of the acoustic startle reflex, have been shown to be highly relevant to the sensory dysregulation described in TS. In fact, PPI deficits are exhibited by TS patients, and are posited to indicate the impaired ability to filter out irrelevant stimuli. Although the primary symptoms of TS are motor and vocal tics, several researchers have pointed out the role of sensory phenomena in the pathogenesis of tics. Sensory experiences affect tics and, in turn, tics are generated in response to intrusive sensory phenomena.

The most commonly prescribed drugs for TS are primarily dopamine antagonists, such as neuroleptics (e.g. haloperidol), benzamides (e.g. sulpiride) or atypical antipsychotics (e.g. risperidone). While finasteride exhibited robust anti-dopaminergic mechanisms similar to those elicited by haloperidol across several behavioral tasks, its effects were not supported by a direct antagonism of dopamine receptors. Accordingly, unlike dopaminergic antagonists, finasteride failed to induce extrapyramidal side-effects. These latter findings are in line with our subsequent studies, in which we analyzed the neurobiological bases of the antipsychotic-like mechanisms of finasteride. We found that the effects of systemic finasteride are mediated by a negative modulation of D_1 (but not D_2) receptors in both rats and mice (Frau et al., 2016, 2013). Specifically, the systemic finasteride administration reverted the PPI deficits produced by the selective D_1 agonist SKF-82958 in Long Evans and C57BL/J mice, rat and mice strains, with higher sensitivity to the effects of dopamine D_1 receptor activation. Furthermore, the fact that finasteride did not counteract the behavioural alterations mediated by the D_2 agonists sumanirole and quinpirole, justifies the lack of catalepsy in both species, even at the higher dose tested.

Our results are particularly relevant in view of recent evidence supporting the therapeutic efficacy of D_1 receptor antagonists in TS. Indeed, by a multicenter, non-randomized, open-label study, Gilbert and co-workers (2014) suggest the pharmacologic antagonism of dopamine D_1 receptors as a novel approach to tic reduction in TS. They found a significant reduction in tic severity, with the selective D_1 antagonist ecopipam and a double-blind trial is ongoing to confirm the efficacy of this pharmacological approach.

Prompted by these preclinical results, we studied the therapeutic potential of finasteride in adult male TS patients. Of note, finasteride, at the same dose used for the treatment of benign prostatic hyperplasia (5 mg/day), led to a gradual improvement of motor and vocal tics in TS patients, as assessed by the Yale Tic Severity Scale, with no reported side effects. Furthermore, the discontinuation of the chronic treatment resulted in an abrupt, dramatic exacerbation of the symptoms, which was countered by the reinstatement of the 5AR inhibitor (Bortolato, Muroni & Marrosu, 2007). Importantly, these preliminary findings have been confirmed in a following open-label study, in which adult male patients show a significant decrease of severity of tics by the sixth week of therapy, with a plateau in the therapeutic effects by the 12th week of finasteride administration. Notably, as in rodents, finasteride did not elicit extrapyramidal side effects in patients (Muroni, Paba, Puligheddu, Marrosu & Bortolato, 2011).

Our preclinical and clinical findings clearly indicated that 5AR plays a key role in the pathophysiology of TS, through the modulation of DA neurotransmission and signalling. In addition, recent preclinical results from our group have suggested that other steroidogenic enzymes might be involved in the pathogenesis of TS. Accordingly, the systemic and intracerebral injections of abiraterone, inhibitor of CYP450 C17, the alternative enzyme responsible of androgens formation, show beneficial effects in animal model of TS, further pointing to the implication of androgen synthesis in TS pathogenesis (Frau et al., 2014). The findings that abiraterone and finasteride elicit similar effects is of particular interest, in view of the convergent neurosteroidogenic pathways and consequent similar substrate and product steroids, and of the translational potential of our results for the clinical practice. Furthermore, it is worth noting that, as reported in TS, the peak age of onset of schizophrenia in males is concomitant with highest testosterone levels at adolescence. In addition, studies in the adolescent striatum and substantia nigra of male rats have shown that testosterone led to increases of tyrosine hydroxylase, D_1-, D_2-, D_5-receptor mRNA and dopamine transporter protein. These data, combined with our previous findings, suggests that the dopaminergic system is upon direct influence of androgen signaling throughout the pubertal/peripubertal period in brain areas with relevance to neurological and neuropsychiatric disorders, including TS and Schizophrenia (Purves-Tyson et al., 2012, 2014). Thus, it is not surprising that imbalances of androgen- or other neurosteroid signaling, specifically during a critical window of brain development, might affect the dopaminergic system. As mentioned above,

a genetic contribution in the etiology of TS has been consistently shown. Although the data from our and other group do not lead to drawn conclusions regarding the functional outcomes, it is feasible that in individuals with an underlying susceptibility for TS, the increase in circulating testosterone at adolescence may serve as trigger for the presentation of dopamine-related tic disorder. In this view, since its role in androgen and neurosteroid synthesis, 5AR may provide a unique biological target in those diseases with male predominance and particular sensitivity to the effects of stress contingencies, in which TS perfectly matches.

Although finasteride is typically well-tolerated, the clinical applications of this drug on TS therapy remain limited; in fact, finasteride cannot be prescribed in children, who represent the broadest target population in this disorder. In addition, finasteride can induce enduring psychoendocrinological side effects in a small subset of patients, including depression and reduction of libido (Traish, Melcangi, Bortolato, Garcia-Segura & Zitzmann, 2015). For these reasons, the identification of the neurobiological bases and molecular mechanisms underlying the effects of finasteride and other 5AR inhibitors, is crucial to overcome their limitations, and develop novel potential therapeutic tools for TS, with limited endocrine side effects.

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Commentary

Structural Analyses in the Study of Anxiety and Anxiety-Related Behaviour

Maurizio Casarrubea[*1]
[1] *Laboratory of Behavioural Physiology, Human Physiology Section "Giuseppe Pagano", Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Italy*

1 Anxiety and Behaviour

According to the latest Diagnostic and Statistical Manual of Mental Disorders, 5th edition, anxiety encompasses various conditions sharing an excessive sense of fear and/or apprehension for no evident reason *and related behavioural disturbances* (Association, 2013). The association of such a gloomy symptomatology, with the great diffusion in the general population, explains the critical impact of anxiety disorders on inter-personal relationships and job-related activities (Greenberg et al., 1999; Wittchen & Hoyer, 2001; Keeley & Storch, 2009). Hence, anxiety disorders represent an important and consistent topic of discussion, not only in terms of underlying neuro-psychological processes but, importantly, also in terms of behavioural dynamics. In such a context, behavioural neurosciences play a central role in understanding anxiety. Different assays are available to study the characteristics of anxiety-related behaviour, such as the open-field (OF), the hole-board (HB) and the Elevated Plus Maze (EPM).

Essentially, an OF consists of an enclosed area where freely moving rodents are observed for a limited period. The OF is commonly employed to study exploration (Drai, Kafkafi, Benjamini, Elmer & Golani, 2001) and anxiety (Choleris, Thomas, Kavaliers & Prato, 2001). The rationale supporting the utilization of the open field in the study of anxiety lies in the natural aversion of rats and mice for novel environments. Indeed, once placed in the OF, rodents spontaneously prefer the periphery, remaining near to the surrounding walls. An increase of time spent in the central zone, increase of the ratio central/total locomotion or the decrease of the latency to enter the central zone represent widely accepted indexes of anxiolysis (Choleris et al., 2001).

The HB is another exploration-based assay, which is well known and commonly used to examine anxiety-related behaviours of rodents (Adamec, Head, Blundell, Burton & Berton, 2006; File & Wardill, 1975a, 1975b; Rodriguez Echandia, Broitman & Foscolo, 1987; Harada et al., 2006; Saitoh et al., 2006; Kalueff, Wheaton & Murphy, 2007; Casarrubea, Sorbera & Crescimanno, 2009b; Kamei et al., 2007). This experimental apparatus generally consists of a square or rectangular arena with a variable number of holes in the ground (Hughes, 2007; File & Wardill, 1975a, 1975b) where the rodent can insert its head. Excluding modified HBs (Ohl, Holsboer & Landgraf, 2001), the presence of the holes represents the essential difference between an OF and a HB. The rationale of the utilization of HB in the study of anxiety classically orbits around the head-dip behaviour. In brief, changes of head-dipping (frequency, latency, duration) are assumed to reflect the anxiety state of the subject: anxiety-inducing drugs decrease both the number and duration of head-dips (Takeda, Tsuji & Matsumiya, 1998), on the other hand, anxiolytic molecules increase head-dips (Takeda et al., 1998).

Finally, the EPM, with thousands of published papers so far, needs little introduction, due to it being the most used experimental assay in the study of anxiety. This apparatus, introduced by Handley and Mithani more than three decades ago (Handley & Mithani, 1984), consists of an elevated plus-shaped platform with two open and two enclosed arms. EPM usefulness has spread towards the understanding of the biological basis of emotionality related to learning and memory, hormones, addiction, and withdrawal (Carobrez & Bertoglio, 2005). The closed arms are surrounded by 50 cm walls and open arms have 0.5 cm edges in order to facilitate entries in the arm (Treit, Menard & Royan, 1993). The rationale underlying the utilization of EPMs in anxiety research is

**Correspondence to*: Maurizio Casarrubea (maurizio.casarrubea@unipa.it)

based on the assumption that rodents will respond to a conflict elicited by the presence of safe parts of the maze (closed and protected), and aversive parts of the maze (open, unprotected and more brightly lit) (Carobrez & Bertoglio, 2005).

2 Assessing the existence of an underlying structure

In general, a structure exists if a set of relationships of any kind can be demonstrated among the components of a given system. Thus, in terms of behavioural analyses, a structure exists only if it is possible to demonstrate the existence of relationships among the activities performed by the subject. It goes without saying that such a demonstration can be extremely challenging if the structure is hidden to the observer, as is the circumstance of animal/human behaviour: as underlined by Eibl-Eibesfeldt, indeed, *"Behaviour consists of patterns in time. Investigations of behaviour deal with sequences that, in contrast to bodily characteristics, are not always visible"* (Eibl-Eibesfeldt, 1970).

The first step is, normally, represented by the construction of a suitable ethogram, that is, a formal description of the components that were taken into consideration. Fig. 1 and Fig. 2 represent ethograms concerning rats' activities in the HB and in EPM respectively. On the basis of the ethogram, video files recorded during experimental sessions need to be observed by means of a suitable software coder, that is, a computer program allowing to record subject's activities in a file. The event log file does represent the essential basis for the all the following analyses.

Hierarchical clustering, stochastic analysis and adjusted residuals analysis are methods based on the elaboration of transition matrices. T-pattern analysis, on the other hand, is a technique able to evaluate the temporal characteristics of sequences of events. Overall, these approaches do represent excellent tools to study behaviour in terms of structural characteristics.

Concerning transition matrix-based approaches, the first step is represented by the construction of a transition matrix (TM) from raw data. Of course, the utilization of a specific software aimed at matrices construction prevents possible errors in TM handling and analysis.

- *Hierarchical Clustering*: the aim of such a procedure is to represent similarities among components, by means of a specific aggregative algorithm. Such a procedure can be either agglomerative (i.e. bottom-up) or divisive (i.e. top-down). Agglomerative bottom-up procedure merges discrete components into successively larger clusters.
- *Stochastic analyses*: the aim of the stochastic techniques is to emphasize probabilistic relationships among behavioural components. On the basis of

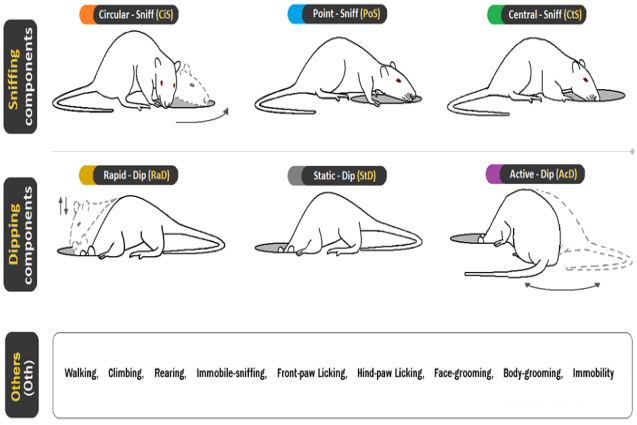


Figure 1: Ethogram of rat behaviour in the hole-board. **Point-Sniff** (PoS) = rat sniffs a single point of the hole-edge; **Circular-Sniff** (CiS) = rat sniffs hole-edge in a continuous circular fashion; **Central-Sniff** (CtS) = rat sniffs hole centre without inserting its head inside; **Rapid-Dip** (RaD) = rat rapidly puts into (eyes no more visible) and removes its head from the hole (no pause between head inserting and removing); **Static-Dip** (StD) = rat puts and maintains its head into hole (eyes no more visible, body maintained in a fixed position); **Active-Dip** (AcD) = rat puts its head into hole (eyes no more visible, body movements produced); **Others** (Oth) = behavioral components not related with hole-exploration. Modified from (Casarrubea, Sorbera, Santangelo & Crescimanno, 2010).

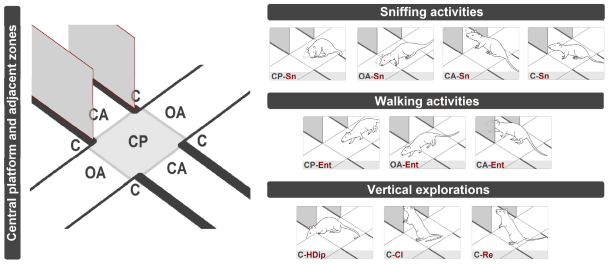


Figure 2: Ethogram of rat behaviour in the central platform of the EPM. Left panel: Only the walls of one closed arm have been represented. CP = Central Platform Area; CA = Closed Arm Zones; OA = Open Arm Zones; C = Corner Zones (closed-open arm junction and external 90° angle comprised between the two arms). Right panel: CP-Sn = Central Platform Sniffing: the rat sniffs the ground of the central platform; OA-Sn = Open Arm Sniffing: the rat sniffs the entrance of one of the two open arms; CA-Sn = Closed Arm Sniffing: the rat sniffs the entrance of one of the two closed arms; C-Sn = Corner Sniffing: the rat sniffs the Plexiglas border of one of the four corners; CP-Ent = Central Platform Entry: the rat moves from an open or from a closed arm to the central platform; OA-Ent = Open Arm-Entry: the rat moves from the central platform to one of the two open arms; CA-Ent = Closed Arm Entry: the rat moves from the central platform to one of the two closed arms; C-HDip = Corner Head Dip: the rat, from one of the four corners, performs scanning head movements in the direction of the floor; C-Cl = Corner Climbing: the rat maintains an erect posture leaning against the Plexiglas border of one of the four corners; C-Re = Corner Rearing: the rat, without leaning against the Plexiglas, maintains an erect posture, facing one of the four corners. Modified from (Casarrubea, Faulisi, Sorbera & Crescimanno, 2015).

the relative frequencies of transitions among the components, a transition matrix can be transformed into a probability matrix.
Four qualifications need to be strictly respected within a probability matrix:

(a) each row must sum 1;
(b) all components must be between 0 and 1;
(c) 0 means no transition between two given components in the originating TM;
(d) switching probability from a component to all others is 1.

– *Adjusted residuals*: an elegant system to assess the significance of cells within matrices has been used by Spruijt and Colleagues (Spruijt & Gispen, 1984) and, after, by different Authors (Casarrubea, Sorbera, Santangelo & Crescimanno, 2010; van den Berg, van Ree & Spruijt, 1999; van Lier, Coenen & Drinkenburg, 2003; Vanderschuren, Spruijt, Hol, Niesink & Van Ree, 1996). Positive residuals indicate transitions occurring more often than expected and negative residuals represent transitions occurring less often than expected. A consistent advantage of adjusted residuals is that they can be expressed according to a Z-distribution, so that p-values can be easily found in a common Z-table and, as a consequence, values $\geq +1.96$ and ≤ -1.96 reveal significant transitions ($p \leq 0.05$).
– *T-patterns detection and analysis*: both in terms of conceptual and procedural aspects, T-pattern analysis is completely different from the above discussed approaches utilizing transition matrices. This analytical technique can be performed using a specific software algorithm, which is able to search for relationships among events in behavioural data by taking into account order, timing, and frequency of these events (Casarrubea, Sorbera, Magnusson & Crescimanno, 2010; Casarrubea, Jonsson et al., 2015; Casarrubea, Faulisi, Magnusson & Crescimanno, 2016). In brief the algorithm compares the distributions of each pair of events (for instance, "A" and "B"), searching for an interval so that, more often than chance expectation, A is followed by B *within* that interval. In a second step, such a T-pattern of first level is considered as a potential starting point for the construction of higher-order t-patterns, e.g., ((A B) C) etc. When no more patterns are found, the search stops (Casarrubea, Jonsson et al., 2015; Casarrubea, Faulisi, Magnusson & Crescimanno, 2016).

3 Modelling the structure: graphical representations

However, independently from the utilized approach, the existence of relationships among the elements of behaviour calls for the possibility to illustrate such relationships. This is an important step. Indeed, transition matrices and related elaborations (similarity, probability and adjusted residuals matrices) are, basically, tables filled with hundreds of numbers. In most cases, a matrix is quite useless in its original form because the meaning of each transition is often difficult to be appreciated. These critical issues are even more amplified with T-pattern analysis, since the detection of T-patterns implies the existence of significant relationships in the course of time. In brief, graphical representations play an essential role in illustrating experimental results.

– *Hierarchical Clustering*: as with the similarity matrix, each cell is representative of a correlation between two given components, on the basis of the reciprocal number of transitions. Hence, a similarity matrix is not a classic "from-to" matrix, but a half-matrix. On the basis of such a half similarity matrix, a dendrogram can be obtained. A dendrogram is a graphical representation showing, by means of a tree structure how much some components are similar one another. An example of a dendrogram, illustrating similarities among behaviours of the rat in the HB, is presented in Fig. 3.

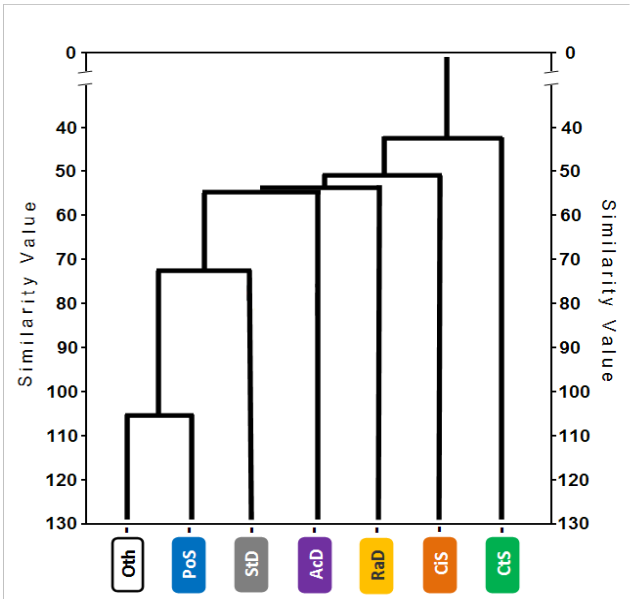


Figure 3: Dendrogram of rat hole exploratory activities representing correlations among behavioural components on the basis of the reciprocal number of transitions. For abbreviations see Fig. 1.

– *Stochastic analyses*: a probability matrix is a normal transition matrix, which can be conveniently expressed through a path diagram where different transition probabilities are represented by connecting arrows of different thickness. Thus, by means of stochastic approach both directions and probabilities among components can be expressed together. Fig. 4 shows, by means of a path diagram, the probabilistic relationships among the behavioural components performed by the animal in the HB.
– *Adjusted residuals*: like probability matrices, adjusted residuals matrices can be illustrated by means of path diagrams. However, this approach might generate, from a conceptual point of view, confounding representations because, if on the one hand, transitions occurring significantly more often than expected can be represented by means of arrows, on the other hand, transitions occurring significantly less often than expected should not. Thus, the representation of positive and negative residuals, by means of histograms, can be profitably employed (Casarrubea, Sorbera & Crescimanno, 2008, 2009b, 2009c, 2009a; Casarrubea, Sorbera, Santangelo & Crescimanno, 2010; Casarrubea, Faulisi, Sorbera & Crescimanno, 2015). Adjusted residuals in Fig. 5 show the association strength of all the detected transitions among the behavioural components performed by the animal in the HB. Positive and negative bars do indicate transitions occurring respectively more and less often than expected.
– *T-Patterns*: T-patterns can be represented by means of tree structures, emphasising significant relationships among events in the course of time. These tree structures, to some extent similar to dendrograms, have the advantage to show patterns distribution along time and, importantly, are very intuitive. The drawback is the huge amount of space required. For instance, concerning results in Fig. 6, overall, 554 patterns do occur in Wistar rats and 792 in DA/Han. In other terms, the illustration of their occurrences by means of the trees illustrated on the right side of Fig. 6, is not an option to be considered. For these reasons we have developed the representation of T-patterns by means of behavioural stripes, that is, the illustration of the onset of each T-pattern, along the x–axis timeline, by means of a simple rasterplot (Casarrubea, Sorbera & Magnusson, M.S Crescimanno, 2011; Casarrubea, Faulisi, Magnusson & Crescimanno, 2016; Casarrubea, Faulisi, Caternicchia et al., 2016).

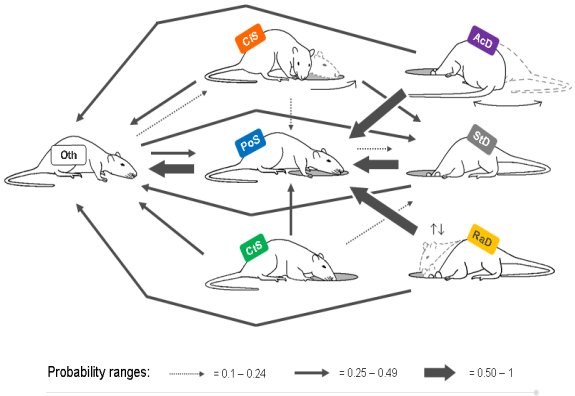


Figure 4: Path diagram representing transition probabilities among behavioural components. Selected probability ranges are indicated at the bottom. For abbreviations see Fig. 1.

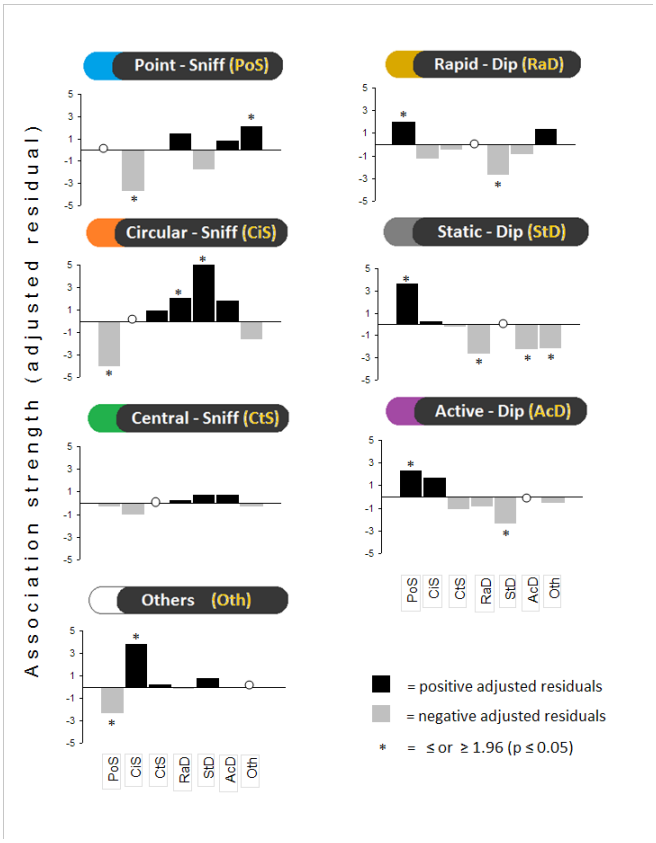
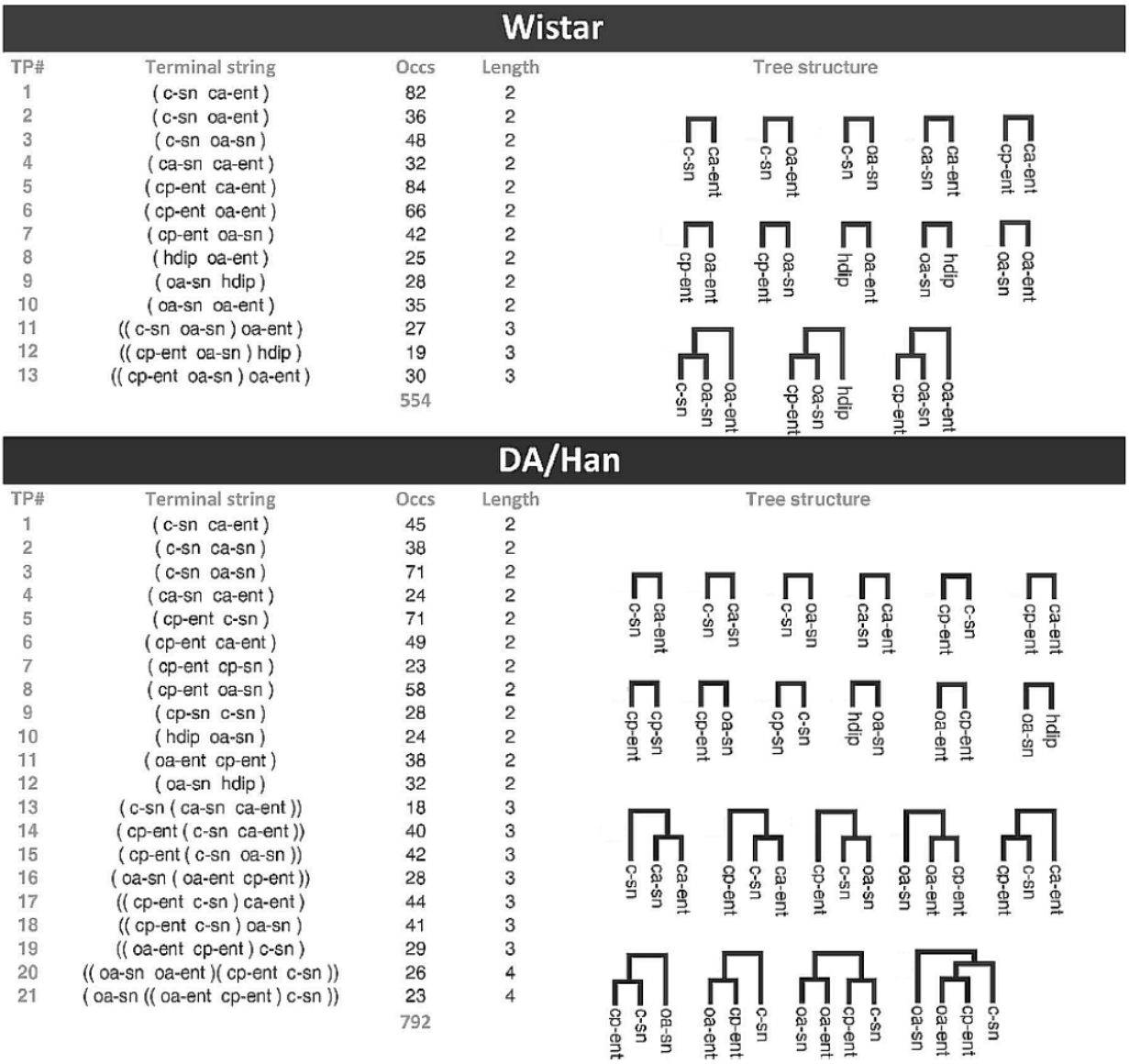


Figure 5: Adjusted residuals. Top of each panel: behavioural elements antecedent to the ones indicated along x-axes. y-axes: adjusted residuals values. Black bars: positive residuals. Grey bars: negative residuals. Empty circles = structural zeroes. According to Z-table, * = significant $p \leq 0.05$ transitions.



Wistar

TP#	Terminal string	Occs	Length
1	(c-sn ca-ent)	82	2
2	(c-sn oa-ent)	36	2
3	(c-sn oa-sn)	48	2
4	(ca-sn ca-ent)	32	2
5	(cp-ent ca-ent)	84	2
6	(cp-ent oa-ent)	66	2
7	(cp-ent oa-sn)	42	2
8	(hdip oa-ent)	25	2
9	(oa-sn hdip)	28	2
10	(oa-sn oa-ent)	35	2
11	((c-sn oa-sn) oa-ent)	27	3
12	((cp-ent oa-sn) hdip)	19	3
13	((cp-ent oa-sn) oa-ent)	30	3
		554	

Tree structure

DA/Han

TP#	Terminal string	Occs	Length
1	(c-sn ca-ent)	45	2
2	(c-sn ca-sn)	38	2
3	(c-sn oa-sn)	71	2
4	(ca-sn ca-ent)	24	2
5	(cp-ent c-sn)	71	2
6	(cp-ent ca-ent)	49	2
7	(cp-ent cp-sn)	23	2
8	(cp-ent oa-sn)	58	2
9	(cp-sn c-sn)	28	2
10	(hdip oa-sn)	24	2
11	(oa-ent cp-ent)	38	2
12	(oa-sn hdip)	32	2
13	(c-sn (ca-sn ca-ent))	18	3
14	(cp-ent (c-sn ca-ent))	40	3
15	(cp-ent (c-sn oa-sn))	42	3
16	(oa-sn (oa-ent cp-ent))	28	3
17	((cp-ent c-sn) ca-ent)	44	3
18	((cp-ent c-sn) oa-sn)	41	3
19	((oa-ent cp-ent) c-sn)	29	3
20	((oa-sn oa-ent)(cp-ent c-sn))	26	4
21	(oa-sn ((oa-ent cp-ent) c-sn))	23	4
		792	

Tree structure

Figure 6: Results of T-pattern analysis in two strains of rats with different emotional reactivity, namely, Wistar and DA/Han strain. TP# = T-pattern identification number;Terminal String = text representation of events in pattern; Occs = number of occurrences of the given pattern; Length = number of events in pattern. Tree structures of detected patterns are illustrated on the right side of the figure. The analysis revealed a very different behavioural structure of Wistar and DA/Han. See Fig. 2 for abbreviations. Modified from (Casarrubea, Faulisi, Magnusson & Crescimanno, 2016).

4 Considerations

Along the last decades, behavioural research on anxiety has been quite conservative in the renewal of their methods. This crucial aspect, examined by several Authors, is not new nor unknown. For instance, Kalueff and co-Workers (Kalueff et al., 2007), discuss the existence of an unfortunate and chronic association consisting of the lack of: a) converging findings and b) new and/or alternative approaches in the study of anxiety and depression disorders. Actually, the largest amount of behavioural studies in the field of psychopharmacology of anxiety and depression have only utilized a small number of descriptive parameters such as latencies, durations and frequencies of individual elements.

Concerning specifically researches on anxiety, several instances demonstrate how quantitative assessments (such as frequencies, latencies, duration of isolated behavioural elements), rarely produce converging findings.

A paradigmatic example, in this sense, is represented by the head-dip. This component, well known to be heavily influenced by changes in rodent's anxiety

level (Takeda et al., 1998; Harada et al., 2006) is, of course, the *"raison d'être"* of the HB assay and, actually, what makes a HB apparatus different from an open-field. Even so, surprising diverging findings do surround this element: in fact increases (Takeda et al., 1998), decreases (Pellow, Chopin, File & Briley, 1985), or no modifications (Sayin, Purali, Ozkan, Altug & Büyükdevrim, 1992) of head-dip frequencies have been described following anti-anxiety drugs administration. Thus, whether head-dip, assessed alone, and disjointed from the whole behavioural structure, is a suitable anxiety indicator, may represent matter of discussion and/or criticism. It is my contention that behaviour is much more than a simple evaluation of disjointed elements through purely quantitative parameters (e.g. durations, frequencies or percent distributions). On one hand, quantitative approaches are useful from a descriptive point of view (since they provide precise information concerning each investigated item). On the other hand, the possibility to characterise each element through even hundreds of numbers does not imply the inverse possibility, namely the possibility to utilize those numbers to reconstruct the behaviour is in its wholeness and, importantly, its meaning (Casarrubea et al., 2009a). As a consequence, descriptive approaches to behavioural studies should be partnered, whenever is possible, with techniques able to provide information on the relationships among the elements of the behaviour. In this sense, transition matrices and T-pattern analyses may represent valuable tools.

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News and Views

Doing Science on a Small Island State: the Challenges for the University and its Surroundings, and the Role of Xjenza

Alfred J. Vella[*1]
[1]*Faculty of Science, University of Malta, Msida, Malta*

"We have the honour to submit to the Government of Malta and to the Royal University the Report which we were invited to prepare. The immediate occasion of our appointment was due to the serious and indeed critical state of the finances of the Royal University. There can be no doubt about the accuracy of that description." (Zarb, 1958).

So begins a 34-page report written by a 5-man commission headed by Sir Hector Hetherington, then Vice Chancellor of the University of Glasgow and Professor of Philosophy, which report is dated 11th September 1957. I was 7 then and in ten years' time I would be about to start my own education at RUM to complete a degree in physics and chemistry.

What has this got to do with the Jubilee Year of Xjenza, you may ask? Let me proceed to read to you some further extracts from the same report and the connection will become clearer.

For context, you will need to remember that at this time, the whole University was sited within this Valletta campus, minus the ground floor, which was being used as a Girls' Secondary School; courses were offered on a biannual basis and the student body was about 300. University "autonomy" had been declared by the Colonial Government 10 years previously by a statute in 1947. The Evans Building, at the bottom of Merchants Street, was still under construction and the lecture rooms and big laboratories were soon expected to, as the report notes, "afford some valuable relief" to the cramped conditions at the St Paul Street site.

As a side note, I cannot help but tell you that I was a Junior College student at this building during 1966–68: I still recall the anatomy department and its cadaver refrigerators in the basement, packed with white to greenish human remains reeking of formaldehyde. This provided a constant opportunity for bravery and bravado to those of us who dared peep inside the fridges or on the chopping tables.

The construction of Evans Laboratories was seen by Hetherington et al. as making it more difficult for the Government to consider building a larger structure for the University, due to the spend on the Evans. Still, they write that "we are certain that, if the University develops as we hope, the accommodation plans now in mind will break down within a very few years... and a good deal of money will have been wasted." This prediction came to pass rather quickly since the foundation stone for a new University was laid at Tal-Qroqq almost exactly 7 years later on 22nd September 1964, when independent Malta was only one-day old, which is 52 years and 2 days ago.

This is what the Commission had to say about the state of Science at the University, and by implication, in Malta as whole:

"It is difficult to describe the present position of science in the University: one can, in effect, say that judged by the standards of most other Universities, science has hardly made more than a beginning. Both in equipment and in the depth of its scientific courses, the University seems to offer little more than is offered by an ordinary secondary school in the United Kingdom. Mathematics gives promise of development. But apart from that, the main present business of the Faculty of Science is to provide a certain amount of basic science preparatory to other degrees. For example, pre-medical sciences (chemistry, physics and biology) are taken in the University. Even there, it seems to us doubtful whether the standard is sufficiently high to form a satisfactory foundation for the much more firmly organized work of the clinical departments. Hence the strengthening of the Faculty of Science appears to us to be one of the main and first concerns of the University of Malta... it is clear that

**Correspondence to*: Alfred J. Vella (alfred.j.vella@um.edu.mt)

it cannot achieve (this aim) unless it is staffed by professors and lecturers who themselves are, fundamentally, trained scientists."

I joined the BSc degree course in 1968: there were 12 of us then and this was (if I recall correctly) the third group that had taken science at University, not as a pre-requisite to medicine or architecture but as an end unto itself. The teaching staff recently employed by Rector Borg Costanzi, were either British academics or freshly graduated Maltese holders of PhD degrees obtained from top UK institutions. All our tutors were trained scientists, not medics or other professionals doing their best to teach mathematics or physics or chemistry without the benefit of a proper grounding in the disciplines. In the short span of 10 years, the University, and indeed, the island nation had turned a page and scientific subjects were finally being studied and taught seriously. This was mainly for purposes such as as secondary school teaching (a hugely important undertaking), working in the hospital laboratories or customs, or for enterprises involved in milk, wine, beer or food making or in drinking water production and so on. Mind you, this was not quite "doing science" as in blue sky researching at the frontiers of the disciplines, but it was providing for Malta, a crucially important corps of competent individuals providing services without which a modern, technically sophisticated nation was never going to make it. Incidentally, the faculty of engineering was established at about this time: this was another important development necessary for the building of a modern society, and it also helped establish the discipline of engineering locally on a scientific, mathematically sound basis.

The teaching and practice of science in Malta suffered a setback, resulting from the ill-conceived suppression of the Faculty of Science (together with that of Arts and Theology) during 1980 to 1987, although some science teaching survived within medicine, engineering and education faculties. The Faculty was fully restored shortly after, now including the new disciplines of computing and IT and with this rebirth, a new development materialized, which may not have been noticed and is rarely, if ever, commented upon: namely, the faculty was re-established with an almost all-Maltese, mostly young, teaching complement who were all scientists!

Xjenza was born from amongst this group of University individuals and their freshly generated cohorts of BSc, MSc and other science-related graduates, nearly all of whom were either in higher studies or busy populating the job market. In the mid 90's, doctoral candidates pursuing work here were still rare on the ground but no longer totally absent.

Serious research in science could only take root in Malta when the University began churning out BSc and MSc graduates in sufficient numbers that at least a few of them would not immediately end up in employment with local manufacturing, industries, the teaching profession or the scientific branches of the civil service or local authorities. Instead they stayed on at the University as research assistants or in doctoral programmes. The numbers involved could not be very large because only about 12% of total undergraduate students follow science, technology, engineering or mathematics (STEM subjects) and most of these are quickly absorbed by employment opportunities where science is a requirement or an added benefit.

The number of students that remain to continue studying and researching as postgraduates are now finally increasing, as they also find another crucial factor, financial support, mainly via government scholarship money that until recently was largely absent. Much as the stipend system was helpful in promoting general tertiary education in Malta, it unwittingly created an anomalous situation where students who may have spent a period dedicated to postgraduate science research at the University were not only not given a stipend, but actually charged a fee for staying on to do this work. So, until very recently, the odds were still stacked against the development of a research culture on the island insofar as the University, a principal actor, was concerned.

Finally we have turned another important page, this time thanks to EU funds, which, through the various scholarship schemes, are channelled towards the support of research activity at the University and in other entities in Malta. This has allowed the indigenous research effort in the physical, biological and engineering sciences to take off within several faculties including science, medicine, dentistry, engineering, built environment and others. It has also helped quell the flow of UM bachelor and master graduates to foreign universities in search of doctoral qualifications from abroad. We also now see inflows of students from foreign institutions looking for research opportunities locally. The Science in the City activity is an excellent occasion to showcase the range and depth of scientific research being pursued locally. What we desperately need is to enthuse as many of our young folk as possible, and to find effective ways and means of propelling them towards science and technology. Science in the City is of course mainly about that. As is the other great experiment happening at Kalkara: namely, Esplora, the interactive science centre, the brainchild of the Malta Council of Science and Technology. May this initiative fire the imagination of as many young minds as possible, drawing them to the marvels of science!

Xjenza has been on the scene during this interesting yet tumultuous period of science in Malta and in my view, it has been an important presence. Even though

seasoned local scientists would tend to turn to the principal high impact journals for publication of their work, Xjenza permits outlets for expression of good research outcomes from early stage researchers. It represents the first credible and durable publication platform in which serious science can find peer-reviewed communication, and especially science which is principally of local interest. Certain papers appearing in Xjenza may possibly be of marginal interest to the international scene, but these same could be of considerable interest locally. For this reason alone, Xjenza deserves to remain permanently on the scene. May the journal serve the local scientific community for many years to come: indeed may it become an important player not only in the dissemination of scientific information by open access, but perhaps even venture into the provision of open science, thus serving as a role model for this practice in the region.

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Xjenza Online - Journal of The Malta Chamber of Scientists
www.xjenza.org

News and Views

20th Anniversary of Xjenza, Science Development in Malta: Role of the MCST

Jeffrey Pullicino Orlando*[1]
[1] *The Malta Council for Science and Technology, Villa Bighi, Kalkara KKR 1320, Malta*

The investments made in modern science and technology have brought us to where we are today. The sheer amount of disruptive innovations, striking discoveries and the multiplication effect of research findings have provided a quantum leap in the advancement of humankind. This, to a magnitude, was not perceivable in the past.

Inspirational advances have been made in health science in terms of stem cell therapy and genome sequencing, in ICT through advanced networking and telecommunications technologies, as well as strong data and supercomputing performance. The energy efficiency and renewable energy sector has opened up new opportunities for societal challenges. In 1959, American physicist Richard Feynman delivered a famous talk entitled "There's Plenty of Room at the Bottom". Since then, nanotechnology advances have broken boundaries within chemistry, biology, physics, materials science and engineering.

Science is an educative process providing technical creativity through inquiry, experimentation and the development of solutions. This is also seen in our local tertiary educational institutions in Malta, maintaining a strong foothold for the significant research being conducted across our islands.

The aforementioned scientific advances are in fact echoed through our local institutions, which have transformed greatly over the years. The research administered through the Malta Council for Science and Technology (MCST) for materials and manufacturing engineering, health and medical devices, renewables and recycling technologies, aviation and aerospace, and the ever-growing projects in ICT, have created a strong portfolio of academic and applied science knowledge. Such research has been conducted with MCST support through the National R&I Programme since 2004. The subject areas of focus evolved from generic thematic areas into the smart specialisation areas MCST has identified, through public consultation, and as presented in the National R&I Strategy 2020.

MCST has not stopped at the development and implementation of National R&I Programmes but - through its other units - has supported numerous researchers through H2020 advice as the National Contact Organisation. H2020 encourages collaborative endeavours with an international dimension, which has vastly developed over recent years. This is particularly important for increased capacity-building and competitiveness on an international scale. Reflecting further on internationalisation, local researchers have come a long way from the limited bi-lateral cooperation with other countries 20 years ago, to a proactive attitude on collaborations with foreign entities. MCST further supports this not only through the medium of EU frameworks, but also through its own Internationalisation Partnerships Awards Scheme. This programme has seen almost 20 research units pairing up with foreign entities to develop excellence in science.

Most science and technology advances are attributed to basic research, technology progression, and the practical implementation in society. Thus, both pure and applied sciences are integral to an attainable scientific solution. MCST understands the importance of economic sustainability and competitiveness through step-change research, in the same way as it is echoed across Europe and beyond. Hence, it has also developed frameworks for supporting researchers in commercialisation feasibility assessment through the programme FUSION. Some 50 local research units have benefitted from guidance and mentoring in this respect. Several of these then proceeded to tap into research funding through a consortium. In fact, since 2004, MCST has disbursed

**Correspondence to*: Jeffrey Pullicino Orlando (jeffrey.pullicino-orlando@gov.mt)

approximately €11 million to its Technology Development Programme.

The R&I ecosystem, which is supported by MCST and other authorities support, has seen a rise in researcher jobs, opportunities for mobility and training, as well as dissemination of research results at international fora. It has observed further interest from the public sector in R&I, to develop products and services that are resourceful for society. In recent years, we have seen a growth in the partnerships between the academic and private (i.e. industrial) sectors, to ensure a smoother transition between the exploratory and basic research, through to technology development and applied solutions for the generation of economic return. The prospects for scientists in Malta, have indeed come a long way.

Despite science being an inspirational subject, its profound mark on virtually everything we encounter in our daily lives is often taken for granted. The relationships between science and everything around us are not always evident to the uninformed. Hence, to propel science popularisation forward and to create an educational basis for scientific inspiration to commence at a young age, MCST have embarked on its largest project yet. The project, Esplora, will help to encourage the uptake of scientific subjects by students who will form tomorrow's future for Malta. This is particularly relevant, as economic sustainability is well-grounded in science education, as well as other factors.

Esplora is a €26 million project designed to create a National Interactive Science Centre. This project, involving the regeneration of a historical site known as The Bighi Naval Hospital, will house a fully interactive and physical experience for students aged between 3 to 15 years old, as well as the general public. The Centre will therefore complement the formal educational system through the promotion of scientific communication, in an informal and interactive manner.

In conclusion, whilst science has developed tremendously over the years, today's achievements and successes should not be seen as end-points, but as way-points to further scientific excellence. The investment in science and research will need to continue at an exponential rate, as new scientific discoveries create an avalanche of further opportunities to be explored and exploited in the future.

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www.xjenza.org

News and Views

The Role of the University of Malta Library in Scholarly Publishing

Kevin J. Ellul*[1]
[1] *University of Malta Library, University of Malta, Msida, MSD 2080, Malta*

Scientists are inevitably tied to publishing. Publish or perish is fundamental dogma in science.

Scholarly communication is bringing innovative discoveries in science, medicine, and other professions into light for readers and scholars. Every sector in our economy and our culture is confronting the pervasive impact of information technology. To this effect, scholarly publishing is right at the centre of the information revolution – from a reliance on print publishing towards online publishing, and an increasing use of other digital forms of scholarly communication, such as scholarly websites and blogs, as well as a growing emphasis on collaborative projects.

This process has been an uneven and even contested one. But the situation is quite clear, with respect to scholarly journals which, given their importance in academia, have received most of the attention. Today, the majority of scholarly online journals are peer reviewed publications that were originally printed publications and migrated, either partly or fully, to an electronic format. Nevertheless, the number of born digital scholarly journals is increasing exponentially.

This transition has a lasting impact on the future direction of scholarly publishing. Subsequently, academic leadership is essential, to ensure that the changes being made provide added value to scholarship, professional expertise, and our quality of life.

As new models of scholarly communication emerge, librarians and information professionals are obligated to play a key role in the development of these models for academic publishing.

Academic libraries have always responded to the needs of faculty and students, by providing adequate scholarly information resources and information retrieval tools, in support of teaching, learning and research. As we moved to the semantic web, the traditional methods and foci of library services changed in ways that demanded libraries to lead in various aspects of the shift in scholarly communication. Indeed, librarians and libraries have already taken on a leading role in transforming the way in which scholarly communication is accessed, stored, preserved, and disseminated. With expertise in copyright law, creative commons licensing, and scholarly communication in general, librarians and information professionals, with solid grounds in the organisation and dissemination of information, have a distinct advantage in assisting scholars in taking control of their intellectual property, disseminating it, sharing it, making it retrievable, and preserving it. These are some of the new pursuits for librarians.

To this effect, the University of Malta Library (UML) has embarked on a number of ambitious projects, which support local researchers with the publishing and dissemination of their research output.

In September 2014, the UML launched the first Institutional Repository (IR) in the Maltese islands, which was branded as OAR@UoM. An IR is a digital platform whereby academics and researchers can upload their research output in Open Access. Subsequently, the scope of implementing OAR@UoM is to collect, preserve and most importantly disseminate the variety of scholarly material that is being produced under the auspices of the University of Malta. Moreover, owing to the fact that OAR@UoM is the sole repository in the Maltese islands, it is also serving as a one-stop resource for national intellectual output and heritage.

OAR@UoM is an OpenAIRE compliant repository, which provides internet-based tools for the submission, processing and uploading of material, whilst possessing its own searching facilities. Nevertheless, since OAR@UoM is integrated with the UML portal, it can also be searched via the Library's discovery and delivery tool *HyDi*. Moreover, the metadata of all content uploaded on the repository is automatically harvested and retrieved via Google and Google Scholar. This will further enhance the visibility, retrieval and dissemination

**Correspondence to*: Kevin J. Ellul (kevin.j.ellul@um.edu.mt)

of the scholarly works uploaded by local researchers and scholars on the IR.

To date, a total of almost 2500 submissions, which include peer reviewed articles, book chapters, conference proceedings, a considerable number of audio recordings and other scholarly content, have been uploaded on OAR@UoM in Open Access.

Amongst the submissions available on OAR@UoM, one can find complete runs of many prominent local scholarly journals, which include the *Malta Medical Journal*, the *Malta Journal of Health Sciences*, the *Bulletin of Entomological Society of Malta*, the *International Journal of Emotional Education*, *Images in Paediatric Cardiology*, *Journal of Malta College of Family Doctors*, *Symposia Melitensia* and *Xjenza Online*.

Open Access to scientific information and research results is a prominent topic in scholarly communication. Subsequently, the UML is actively contributing towards Open Access initiatives. Following the implementation of OAR@UoM, the UML recognised the need to draft an Institutional Open Access Policy for the University of Malta. The scope of this policy is to:

- further support academic publishing in Open Access,
- guarantee that any research output produced under the auspices of the University is made available in Open Access,
- support researchers to adhere with the H2020 mandates,
- facilitate wider dissemination, and
- enhance the visibility of local academics and the University at large.

The UML's objective for developing an Institutional Open Access Policy is to have a rights-retention instrument, which grants a non-exclusive license or permission to upload scholarly peer reviewed research papers on the University's IR in Open Access. Moreover, this policy will enable researchers and scientists to retain copyright ownership of their scholarly works and research output.

To this effect, the UML has compiled a draft policy which recommends self-archiving, that is, Green Route OA, while supporting Open Access publishing that is the Gold Route. Following the implementation of an Open Access policy for the University of Malta, the Library will be supporting MCST with the development of a National Open Access Policy.

Throughout the last few years, the UML has also actively participated in two pan-European projects, known as OpenAIRE2020 and PASTEUR4OA. OpenAIRE2020 aims to establish an open and sustainable scholarly communication infrastructure for research in Europe, while the remit of PASTEUR4OA is to support the European Commission's recommendation to member states to develop and implement policies, to ensure Open Access to all research outputs from publicly funded research. Additionally, the UML also acts as a National Point of Reference for access to and preservation of scientific information in Europe.

To further support local researchers, the UML organises ongoing Author Workshops, whereby prominent publishers deliver onsite training sessions to equip local scholars with advice and guidance on how to publish their research output. The scope of these sessions is to provide an insight into choosing the appropriate scientific journals for their articles; why editors accept or reject research papers; how to construct a research paper; an evaluation of the review process; an understanding of the importance of ethical issues and tips on how to enhance the visibility of the publication. These workshops are beneficial for both early careers and established researchers.

To assist local editors with the publishing of scholarly journals, the UML is in the process of implementing a Journal Management System. This software is designed to facilitate the development of peer reviewed publishing. It provides the technical infrastructure for online presentation, as well as the entire editorial management workflow, including article submissions, multiple rounds of peer reviewing, indexing and publishing.

In conclusion, the emergent modes of scholarly communication are charting important territories in the rapidly changing information landscape. Publishing of scholarly research propels science forward, paves the way to innovation, and thus helps provide solutions to the problems being faced by society. In this regard, there is a real opportunity for academia to take advantage of the momentum for change in scholarly communication. This is the time to exploit the various publishing models and to join efforts, eliminating barriers that hinder dissemination of scientific research.

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www.xjenza.org

Proceedings of Science in the House

5th Annual *Science in the House* Exhibition

David C. Magri[*1]

Celebrating its 5th anniversary, this year's *Science in the House* exhibition was a most memorable occasion. The event was held on Thursday 29th September at 11:00 am in the main foyer of the Parliament Building in Valletta. The event commenced with opening remarks from Prof. Alex Felice, chairman of the *Science in the City/European Researchers' Night* consortium followed by a distinguished list of speakers including Prof. Alfred Vella, Rector of the University of Malta, the Hon. Evarist Bartolo M.P., Minister for Education and Employment, and the Hon. Anġlu Farrugia M.P., Speaker of the House of Representatives, Parliament of Malta.

This year's event showcased a selection of research projects on neuroscience and brain research in addition to the regular compliment of posters from across various research disciplines. A set of four posters on the neuroscience theme presented research on the use of stereo-electroencephalography (EEG), the treatment of epilepsy with light, biochemical studies of autism and research related to depression, addition and epilepsy. These studies involve collaborations between Prof. Giuseppe Di Giovanni and colleagues from other institutions including Prof. Giacomo Rizzolatti, Dr Fausto Caruana, Prof. Vincenzo Crunelli and Dr Maria Cristina D'Adamo.

The University of Malta is fortunate to have a deep roster of neuroscience and brain research experts. Another column of four posters showcased projects by Prof. Neville Vassallo, Prof. Mario Valentino, Dr Ruben Cauchi and Prof. Richard Muscat on the discovery of new drugs for Alzheimer's and Parkinson's diseases, *in vivo* imaging of astrocytes, the use of genetically modified fruit flies for studying motor neuron disease and the study of neural brain function and oscillation, respectively.

New this year, biographical posters of 4 Maltese scholars from the past and present who have contributed significantly to the field of cognitive science and neuroscience were part of the opening ceremony. The biographical posters of Prof. Louis Vassallo (medicine), Prof. George Xuereb (pathology, former Rector), Prof. Edward de Bono (inventor of the concept of lateral thinking) and Prof. Ludwig Zrinzo (neurosurgery) were accompanied by the physical presence of Prof. de Bono and Prof. Zrinzo. Family members of the late Prof. Vassallo and late Prof. George Xuereb were also in attendance for the ceremony.

Representation of various research projects came from the Faculties of Science, Medicine and Surgery, Health Sciences and Economics, Management & Accountancy. Topics ranged from electromagnetic fields in medicine, evaluation of earthquake risk on the Maltese Islands, the economics and politics of household recycling, zebrafish as an animal model for studying bone diseases, novel hybrid optomechanical technologies, human foraging and attention, yeast cell cycle studies with aspirin and genetic studies of type II diabetes. In addition, this year the exhibition also included posters from four research-oriented organisations: Malta Life Science Park; Malta College of Arts Science and Technology (MCAST); Research, Innovation and Development Trust (RIDT) and the Malta Medicines Authority.

The exhibition remained on display for public viewing the following Friday and Saturday evenings for the *Science in the City/European Researchers' Night* and *Notte Bianca* festivals, respectively. The exhibition was left on display for the general public and parliamentarians for another week afterwards.

Science in the House is organised by the Malta Chamber of Scientists, the RIDT and the *Science in the City/European Researchers' Night* consortium and part funded by the EU Marie Skłodowska-Curie Action of the Horizon 2020 (H2020) Programme. More information can be found at www.scienceinthecity.org.mt or at www.facebook.com/ScienceInTheCityMalta.

Correspondence to: David C. Magri (david.magri@um.edu.mt)

Figure 1: Guests during the opening remarks of *Science in the House* during the speech of the Hon. Evarist Bartolo. In the front row from left to right are Prof. Alfred Vella, the Hon. Anġlu Farrugia, Speaker of the House, the Hon. Helena Dalli and Prof. Alex Felice and the Hon. Chris Agius (second row to the right).

Figure 2: The main foyer of the Parliament Building showcasing the exhibition before the commencement of the 5th annual *Science in the House* exhibition.

2016 *Science in the House* Posters by Title and Contributing Researchers

1. Electromagnetic Fields in Medicine – Prof. Charles Sammut, Prof. Pierre Schembri-Wismayer, Dr Lourdes Farrugia, Iman Farhat, Julian Bonello
2. Evaluation of Earthquake Risk on the Maltese Islands – Prof. Pauline Galea, Dr Sebastiano D'Amico, George Bozionelo, Daniela Farrugia
3. Human Foraging and Attention – Prof. Ian Thornton, Marcello Gómez Maureira, Marthese Borg, Amanda Muscat
4. Zebrafish as an Animal Model for Studying Bone Diseases – Prof. Angela Xuereb, Dr Melissa Formosa
5. The Economics and Politics of Voluntary Household Recycling – Dr Marie Briguglio
6. Hybrid Optomechanical Technologies – Dr André Xuereb, Vittorio Peano
7. The Genetic Epidemiology of Type 2 Diabetes – Prof. Josanne Vassallo, Dr Nikolai Pace, Seham Eljali
8. The Cell Cycle is Crucial for Cell Survival and Death – Prof. Rena Balzan, Dr Gianluca Farrugia, Maria Azzopardi
9. Discovering New Drugs for Alzheimer's and Parkinson's Diseases – Dr Neville Vassallo, Stephanie Ghio
10. *In Vivo* Imaging and Monitoring Astrocytes in Health and Disease – Prof. Mario Valentino, Dr Christian Zammit, Robert Zammit
11. Genetically Modified Fruit Flies to Untangle Mysteries of Motor Neuron Disease – Dr Ruben Cauchi, Michelle Briffa, Rebecca Cacciottolo, Rebecca Borg
12. Brain Function and Neural Oscillations – Prof. Richard Muscat, Nowell Zammit
13. Decomposing tool action observation by sEEG – Prof. Giacomo Rizzolatti, Dr Fausto Caruana
14. Treating Epilepsy with Light – Prof. Vincenzo Crunelli
15. Autism Spectrum Disorder with Intellectual Disability – Dr Maria Cristina D'Adamo
16. Curing Depression, Addiction and Epilepsy – Prof. Giuseppe Di Giovanni
17. Malta Life Sciences Park – Dr Joseph Sammut
18. MCAST Water Research and Training Centre – Dr Alex Rizzo
19. Research, Innovation and Development Trust (RIDT) – Wilfred Kenely
20. Malta Medicines Authority – Prof. Anthony Serracino Inglott

Gratitude is extended to C. Camilleri & Sons for catering, Pierre Mallia, Director, House of Representatives, and Ryan Cutajar and Gianni Zammit of JugsMalta for logistic support, Siobhan Vassallo for the poster design, Angele Galea for assistance with the biography poster exhibition and individuals who contributed to the institution posters.

Figure 3: Researchers Lourdes Farrugia (left) and Julian Bonello (second from left), Dr Leonie Baldacchino (middle left) speaking with Prof. Alex Felice (middle right), Hon. Anġlu Farrugia (second from right) and Prof. Charles Sammut, Dean of the Faculty of Science (right).

ELECTROMAGNETIC FIELDS IN MEDICINE

OBJECTIVES OF THE RESEARCH

High-frequency electromagnetic fields (EMF) are used to study the human body, in particular the water content in various tissues. Using EMFs we can distinguish cancer tumours from healthy tissue as the amount of water in tumours is higher than in healthy tissue. The difference in the amount of EMF energy absorbed by healthy and cancerous cells allows for therapeutic and diagnostic applications.

MAIN FINDINGS TO DATE

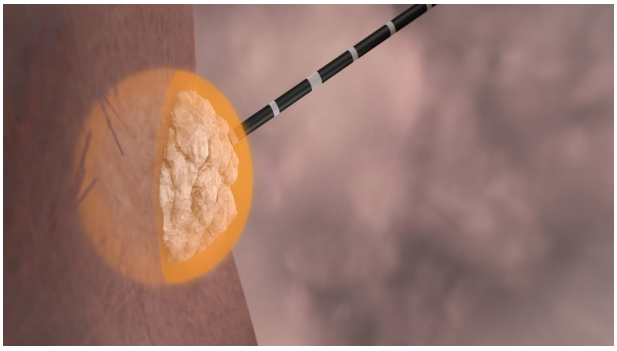
The electrical properties of liver, kidney and muscle tissue are dependent on the tissue age. A recent study showed that the properties of the liver measured in vivo can be estimated from ex vivo measurements. Liquids mimicking the electrical properties of different organs have been formulated.

SOCIO-ECONOMIC IMPACT

Computational and experimental methods have provided insight into the effect EMF has with different organs and tissues in the human body. This research could lead to the development of new medical devices and more effective treatments in oncology.

THE RESEARCH TEAM

The research is led by Prof. Charles Sammut of the Department of Physics in collaboration with Prof. Pierre Schembri-Wismayer of the Department of Anatomy, Dr Lourdes Farrugia of the Department of Physics and postgraduate students Julian Bonello and Maik Pertermann at the University of Malta.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

The research is supported by the University of Malta and the European Regional Development Fund (ERDF) grants 018 and 310. The research group is involved with various COST Actions and collaborates with the National University of Ireland in Galway, the Energy and Economic Development (ENEA) in Rome and Supelec, University Paris-Sud in France.

EVALUATION OF EARTHQUAKE RISK ON THE MALTESE ISLANDS

OBJECTIVES OF THE RESEARCH

Historical and geological studies indicate that the possibility of serious earthquake damage to the Maltese islands is real. Our research aims to evaluate the threat and potential damage that could result from earthquakes. Using state-of-the-art seismic monitoring systems and virtual regional seismic networks, we are investigating known and newly-discovered tectonic features. New imaging techniques provide a way of determining the site-specific amplification of incoming seismic waves and hence the surface ground response. The data is used to generate realistic earthquake ground motion scenarios for risk-assessment purposes.

MAIN FINDINGS TO DATE

Our research has revealed that the frequency of earthquakes around the Maltese islands is much greater than previously thought. New instrumentation provides increased sensitivity for detecting tremors. We have also identified the role that clay layers have on site- and frequency-dependent seismic amplification.

SOCIO-ECONOMIC IMPACT

Through collaboration with the civil engineering community, this research will influence national strategies for designing and implementing improved construction guidelines for safe buildings that take into account the specific local geology. A holistic earthquake risk assessment will also provide important inputs to the insurance industry and Civil Protection.

THE RESEARCH TEAM

The research is carried out by the Seismic Monitoring and Research Group within the Department of Geosciences coordinated by Dr Pauline Galea with team members Dr Sebastiano D'Amico, George Bozionelos, Daniela Farrugia, and Dr Matthew Agius in collaboration with a number of academic institutions in Europe and America.

ACKNOWLEDGMENT OF RESEARCH SUPPORT

Financial support has come from the SIMIT project (B1-2.19/11, Italia-Malta OP 2007-2013); Endeavour scholarship; ERDF 310 (Expanding the Physics and Applied Interdisciplinary Research Capabilities at the Faculty of Science), COST Actions ES1401, ES1301, TU1208 and the University of Malta.

HUMAN FORAGING AND ATTENTION

OBJECTIVES OF THE RESEARCH

The objective is to use a novel approach to understanding how human perception and action is constrained by attention. Inspired by classic animal foraging research, we have developed a series of mobile phone apps that are used to collect data from diverse groups of human participants in a variety of settings. Here, we present some of our main findings and provide demonstrations of our tasks with particular focus on a new 3D scenario developed as a collaboration between the Department of Cognitive Science and the Institute of Digital Games.

MAIN FINDINGS TO DATE

Our studies suggest that human foraging is tightly constrained by attention. Search is flexible when individual items can be easily detected, but becomes more limited when viewing conditions deteriorate or attentional load increases. However, some individuals ("super-foragers") do not conform to this pattern. Their behaviour raises important questions about current theories of attention.

SOCIO-ECONOMIC IMPACT

Understanding and improving how we search has implications in many areas including consumer behaviour, medical image interpretation and airport security screening. We are constantly asked to divide our attention (e.g., talking and driving). Knowing when we can and cannot do this could save lives. How people control attention could shed light on several deficits that affect patient populations.

THE RESEARCH TEAM

The project is led by Prof. Ian Thornton of the University of Malta and Prof. Árni Kristjánsson of the University of Iceland, and assisted by two post-doctorate researchers based in Iceland, Ómar Jóhannesson and Andrey Chetverikov. In Malta, Francesca Borg Taylor-East, Elizabeth Camilleri & Isabelle Kniestedt at the Institute of Digital Games have developed a new foraging app.

ACKNOWLEDGMENT OF RESEARCH SUPPORT

Financial support for this project comes from the University of Malta, the Icelandic Research Fund (Rannís), the Research Fund of the University of Iceland, the Russian Foundation for Basic Research and the Saint Petersburg State University.

ZEBRAFISH AS AN ANIMAL MODEL FOR STUDYING BONE DISEASES

OBJECTIVES OF THE RESEARCH

Osteoporosis is a skeletal disease whereby bones become weaker and fragile resulting in increased risk of fractures. The disease can be caused by both lifestyle factors and genetic traits. Individuals with a family history of osteoporosis are prone to becoming affected at an early age. Using zebrafish as an animal model, the genes that cause osteoporosis are being assessed for their effect on bone development and cartilage formation.

MAIN FINDINGS TO DATE

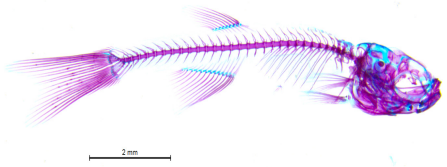
Around 80% of the zebrafish genes are identical to those of humans, making them a good animal model. Therefore, zebrafish can be used to study defects in bone and cartilage formation. Genetic studies on Maltese families with multiple affected members have led to the identification of new genes that are hypothesised to affect bone development.

SOCIO-ECONOMIC IMPACT

Genetic factors play a major role in the susceptibility to osteoporosis. Identifying these genetic factors could aid in the early diagnosis of osteoporosis and the development of personalised medicine. Preventive measures and early detection could prevent unnecessary suffering in high-risk individuals.

THE RESEARCH TEAM

The principal investigators are Prof. Angela Xuereb-Anastasi and Dr Melissa Formosa from the University of Malta. The research is conducted in collaboration with Prof. André Uitterlinden, Prof. Fernando Rivadeneira, Dr Annemieke Verkerk, and Prof. Rob Willemsen from the Erasmus University Medical Centre in Rotterdam.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

The research is supported by the Malta Community Chest Fund in conjunction with the Research Innovation and Development Trust (RIDT).

THE ECONOMICS AND POLITICS OF VOLUNTARY HOUSEHOLD RECYCLING

OBJECTIVES OF THE RESEARCH

Municipal solid waste is a by-product of economic production and consumption. Its management and mismanagement incurs high capital and running costs, creates environmental and health impacts. A remedy imposed in several countries is a (polluter pays) tax on household waste disposal. However, the introduction or an increase in taxes is politically unpopular – sometimes stimulating illegal disposal. Communication campaigns to encourage voluntary cooperation could be a solution, although little economic research data is available in this field.

MAIN FINDINGS TO DATE

Our findings show that households are willing to recycle voluntarily. Household conditions (like space and time) and intervention characteristics (like convenience) stimulate voluntary uptake. We have discovered that political preferences influence household willingness to cooperate. When there is a negative sentiment towards the party in government, households are less willing to participate in government sponsored schemes.

SOCIO-ECONOMIC IMPACT

This research helps policy-makers and scheme-operators to increase household participation in recycling. Voluntary cooperation can cultivate public-spirited motives and lower the administrative, environmental and political costs of compliance. The finding that political preferences influence cooperation will result in better ways in which a public-good scheme is communicated to the public and help forecast uptake.

THE RESEARCH TEAM

The research team consists of Dr Marie Briguglio of the Department of Economics at the University of Malta and Prof. Liam Delaney and Prof. Alex Wood of the University of Stirling in the UK.

ACKNOWLEDGMENT OF RESEARCH SUPPORT

The research was conducted at the University of Stirling and part-funded by an Early Career Engagement Grant of the Scottish Institute for Research in Economics (SIRE) and PhD bursary from the University of Malta.

HYBRID OPTOMECHANICAL TECHNOLOGIES

OBJECTIVES OF THE RESEARCH

Hybrid Optomechanical Technologies (HOT) are a new kind of sensing and information processing technology based on light and motion. Scientists know that light, just like water emerging from a hosepipe, can push small things around. This concept can be used to control the motion of small objects with light and to measure their position very accurately. New devices will be developed that use light and motion to store or route information, or make extremely accurate measurements.

MAIN FINDINGS TO DATE

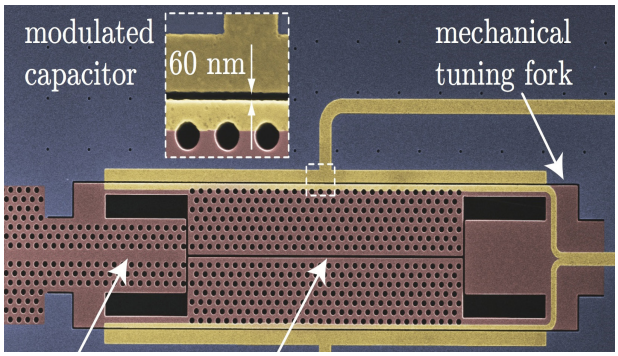
The consortium has demonstrated how to use tiny moving objects to produce diodes for light. Such devices allow optical signals to go in one direction from A to B, but not vice versa. These technologies may be used to amplify or process optical signals inside the computer chips of the future. We have built devices that demonstrate quantum entanglement in the vibrations of two microscopic drums. This will answer the question of why ordinary objects, like human beings, never seem to behave quantum mechanically, like being in two places at once.

SOCIO-ECONOMIC IMPACT

Completely new information processing technologies must be developed for computers to keep getting smaller and faster. Technologies using light instead of electricity are promising candidates. However, researchers are unsure how ordinary computer chips, which use electrical signals, can be developed to process light directly. HOT may solve this problem with new technologies that are faster and more energy efficient than those currently used today.

THE RESEARCH TEAM

The research team at the University of Malta consists of the Quantum Research Group within the Department of Physics in the Faculty of Science. The project is led by Dr André Xuereb. The project involves a consortium of 16 other institutions including several leading research team from European universities and four industrial partners (IBM, Hitachi, ST Microelectronics, and Thales SA).



ACKNOWLEDGMENT OF RESEARCH SUPPORT

HOT is supported by the Horizon 2020 programme of the European Commission. It builds on work supported through several private and public research initiatives, including the FP7 programme, and COST Action networks. We also acknowledge funding from RIDT and MCST.

THE GENETIC EPIDEMIOLOGY OF TYPE 2 DIABETES IN MALTA

OBJECTIVES OF THE RESEARCH

Type 2 diabetes is a metabolic disease characterised by high blood sugar levels in patients. The disease is a major risk factor for the development of cardiovascular disease and eventually death. The objective of our research is to explore the relationship between select genetic variants and the risk of diabetes. We examine DNA from new born babies as a reference and compare healthy senior citizens without diabetes to those with diabetes in the Maltese population. Patients from Malta and Libya are compared to explore the relationship between genetics and clinical traits.

MAIN FINDINGS TO DATE

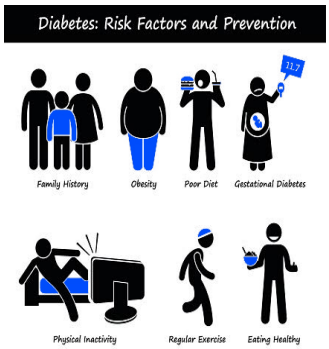
We have discovered that there are eight candidate genes consistently association with occurrence of type 2 diabetes in the Maltese population. These gene variants serve functional roles in inflammation, fat cell development and energy expenditure. Specific subgroups with diabetes were used for this study to identify any association between genetic makeup and body composition.

SOCIO-ECONOMIC IMPACT

The genetic findings could be potentially used for the early identification of individuals at risk of developing type 2 diabetes allowing for preventive measures and personalised treatment strategies.

THE RESEARCH TEAM

The research team consists of Prof. Josanne Vassallo and Prof. Alex Felice and researchers Dr Nikolai Pace and Seham Eljali of the Faculty of Medicine & Surgery at the University of Malta.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

This work is part financed by the Strategic Educational Pathways Scholarship scheme grant and by the University of Malta.

THE CELL CYCLE IS CRUCIAL FOR CELL SURVIVAL AND DEATH

OBJECTIVES OF THE RESEARCH

The immediate objective of our research work is a better understanding of cell-cycle involvement in cell survival or programmed cell death. A number of genes which are involved in both cell survival and death have been identified, most of which play a role at cell-cycle checkpoints. Checkpoints ensure that one phase of the cell cycle is complete before a subsequent phase starts, thereby guaranteeing that the daughter cells are genetically identical to their parent cell. If checkpoints are non-functional, the cell cycle will proceed with damaged DNA, giving rise to defective daughter cells. We use yeast cells as experimental models of higher organisms to study aspirin's effect on oxidative stress and programmed cell death.

MAIN FINDINGS TO DATE

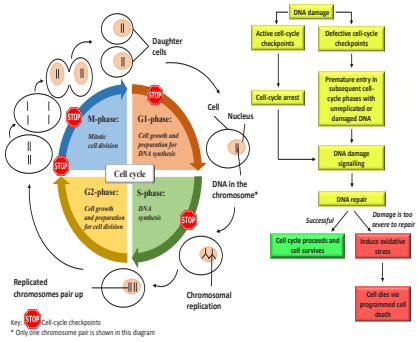
Our research team has observed that our redox-compromised yeast cells, which resemble cancer cells, die by programmed cell death in the presence of aspirin unlike normal, healthy yeast cells. Our studies to date showed key aspirin-induced differences in the physiology of the cells between these two sets of yeast cells.

SOCIO-ECONOMIC IMPACT

Aspirin is highly-prescribed to prevent cardiovascular events. However, its preventive anti-tumour properties are still not fully understood. Thus, our studies help to better understand the mechanisms by which aspirin distinguishes between cancer and normal cells and selectively targets cancer cells. The studies may allow for the development of better aspirin-like drugs or novel anti-cancer therapies.

THE RESEARCH TEAM

The research team consists of the project leader Prof. Rena Balzan, Research Support Officer III Dr. Gianluca Farrugia and Ph.D. student Ms. Maria Azzopardi. Institutions contributing to this research work include the Genomic's Core Facility at the European Molecular Biology Laboratory (EMBL) at Heidelberg, Germany and the Institute for Molecular Biosciences, Karl-Franzens University of Graz in Austria.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

This work is part of project R&I-2015- 001, which is financed by the Malta Council for Science & Technology through the R&I Technology Development Programme.

DISCOVERING NEW DRUGS FOR ALZHEIMER'S AND PARKINSON'S DISEASES

OBJECTIVES OF THE RESEARCH

Alzheimer's disease (AD) and Parkinson's disease (PD) are devastating illnesses that are currently incurable. Combined they afflict around 8 million people in Europe: AD leads to severe memory loss (dementia) whilst PD results in uncontrollable tremors and slowness of movement. The objective is to discover new drugs for the prevention and/or treatment of these neurodegenerative maladies. Specifically, we seek to block a key molecular interaction between toxic aggregates and mitochondria, the so-called 'powerhouses' of brain cells.

MAIN FINDINGS TO DATE

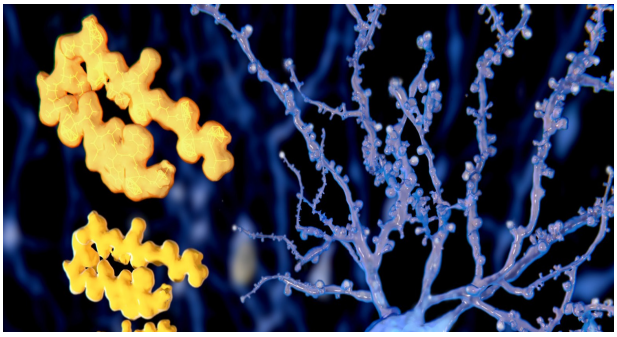
We have shown that toxic amyloid aggregates that form in the brains of AD/PD patients damage the mitochondria of brain cells by attacking a lipid molecule known as cardiolipin in the mitochondria's double-membrane. Furthermore, we have identified 3 naturally-occurring compounds and a marine plant extract that exhibit strong anti-aggregate activity for preventing mitochondrial damage.

SOCIO-ECONOMIC IMPACT

Our research could result in the discovery of new therapeutic strategies for preventing or slowing the progression of Alzheimer's and Parkinson's disease. A new effective treatment could improve the quality of life of millions of patients, and alleviate the burden on caregivers.

THE RESEARCH TEAM

The research team is led by Dr Neville Vassallo in collaboration with Dr Ruben Cauchi of the Department of Physiology & Biochemistry and the Centre for Molecular Medicine and Biobanking of the University of Malta. Current team members are Dr Mario Caruana, Michelle Briffa, Angelique Camilleri and Stephanie Ghio. Past team members include research students Alison Gauci, Claire Zarb, Dr Johanna Neuner and Ulrike Ostermeier of the University of Munich, Germany.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

The research has been supported by two RTDI programs from the Malta Council of Science & Technology (R&I-2008-068 'NEUROAMYLOID' and R&I-2012-066 'MODiFLY'). Additional funding has been provided by the University of Malta (PHBR-06) and the Faculty of Medicine & Surgery (MDSIN08-21).

IN VIVO IMAGING AND MONITORING ASTROCYTES IN HEALTH AND DISEASE

OBJECTIVES OF THE RESEARCH

Stroke is the third most common cause of death in men and the second most common in women according to the World Health Organization (WHO). In Malta 10.4% of all deaths were due to stroke. It is also the most common cause of severe disability with 1 in 4 men and 1 in 5 women expected to have a stroke by age 85. Current treatment options are extremely limited. Hence, there is a great need for new treatment strategies. Our goal is to better understand the mechanisms of astrocyte function in order to develop new treatments for stroke.

MAIN FINDINGS TO DATE

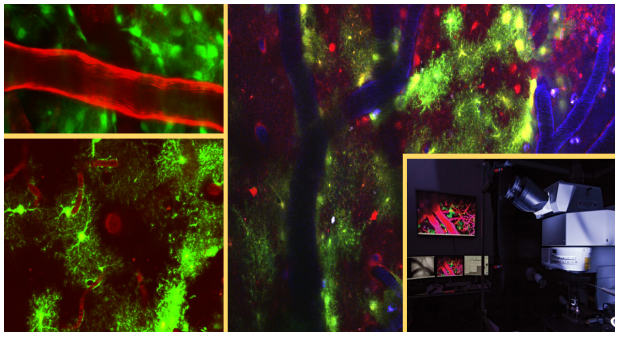
Brain function is maintained by an integrated system consisting of specialised cells called astrocytes. Contrary to published reports, we show that astrocytes are more vulnerable to injury than previously reported using new laser imaging techniques with improved resolution to study the brain *in vivo*. We have discovered that there are subclasses of astrocytes within the same region of the brain exhibiting different vulnerabilities to stroke.

SOCIO-ECONOMIC IMPACT

Despite major advances in prevention and rehabilitation, few neurological injuries are as debilitating as stroke. Our research is providing new insight into astrocytes and could result in more effective treatment.

THE RESEARCH TEAM

The stroke research team consists of Prof. Mario Valentino (Department of Physiology & Biochemistry) and researchers Dr Christian Zammit, Jasmine Vella and Robert Zammit in collaboration with Prof. Robert Fern and exchange students from the University of Plymouth.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

The research is financially supported by the University of Malta Research and through collaborations with the University of Plymouth part-funded by the Research Councils UK.

GENETICALLY MODIFIED FRUIT FLIES FOR STUDYING MOTOR NEURON DISEASE

OBJECTIVES OF THE RESEARCH

Spinal Muscular Atrophy (SMA) and Amyotrophic Lateral Sclerosis (ALS) are motor neuron diseases that deprive patients of their ability to walk, eat or breathe. While ALS occurs in adults, SMA affects young children. Both SMA and ALS patients have a deficiency of the Survival Motor Neuron (SMN) protein that results in the death of motor neurons and the associated muscles. Using the fruit fly Drosophila melanogaster as a model organism, we are learning how the SMN protein works in the neuromuscular system of living organisms.

MAIN FINDINGS TO DATE

The SMN protein is involved in the assembly that cuts and pastes the cell's genetic instructions together. We discovered that disruption of this function leads to the collapse of the neuromuscular system of fruit flies in a similar manner to SMN deficiency. This breakthrough discovery implies that failure to correctly process the genetic blueprint for the production of functional proteins could be the reason for the neuromuscular deficits in SMA or ALS.

SOCIO-ECONOMIC IMPACT

Current therapies for SMA or ALS are based on boosting SMN protein levels. Broadening the therapeutic targets is essential for an effective treatment. Model organisms such as the fruit fly are key tools for the successful implementation of this strategy.

THE RESEARCH TEAM

The team is led by Dr Ruben J. Cauchi and postgraduate students Michelle Briffa and Rebecca Borg and undergraduate students Maia Lanfranco, Benji Fenech Salerno and Rebecca Cacciottolo in collaboration with Dr. Neville Vassallo's research group, the French National Centre for Scientific Research (CNRS) and the Institute of Cellular Pharmacology Ltd.

ACKNOWLEDGMENT OF RESEARCH SUPPORT

This research is supported by the University of Malta, the Faculty of Medicine & Surgery, and the Malta Council for Science & Technology (MCST).

BRAIN FUNCTION AND NEURAL OSCILLATIONS

OBJECTIVES OF THE RESEARCH

One of the most remarkable dimensions of humankind is the brain. The brain is responsible for both our normal healthy and unhealthy behaviour. This tempts one to ask the question: but how? The brain is composed of billions of 'micro' power stations, called neurons that generate complex electrical activity patterns. In our studies, we are attempting to understand behaviour and its abnormal facets in neurodevelopmental conditions, such as ADHD, by analysing the brain electrical activity called neural oscillations. This research will further our understanding of the neural basis that sub-serves ADHD, and provide insight into the therapeutic effects elicited by psychostimulant medication.

MAIN FINDINGS TO DATE

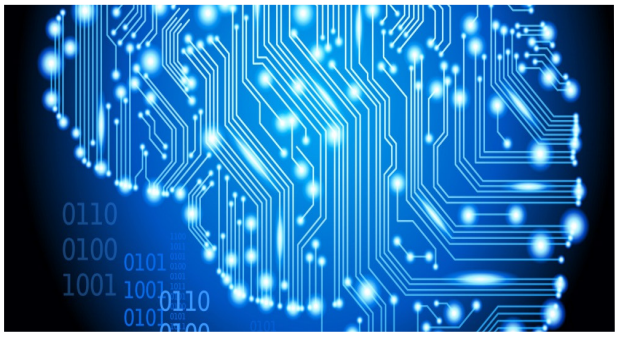
We have discovered that neural oscillations are crucial during mental process involved in understanding or the senses and that abnormal neural oscillation can distinguish between ADHD and healthy subjects. Furthermore, we have learned that psychostimulant medication improves the abnormal neural oscillations in ADHD.

SOCIO-ECONOMIC IMPACT

We envision that brain signals can be used as objective biomarkers to diagnose ADHD rather than 'paper and pencil' clinical questionnaires. In the future, brain signals may be used to assist practitioners in the administration of psychostimulant medication.

THE RESEARCH TEAM

The research team consists of Prof. Richard Muscat & Dr Nowell Zammit and colleagues at the Centre for Biomedical Cybernetics at the University of Malta.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

The research is financially supported by the Department of Physiology and Biochemistry of the Faculty of Medicine and Surgery at the University of Malta.

DECOMPOSING TOOL ACTION OBSERVATION BY sEEG

OBJECTIVES OF THE RESEARCH

A fundamental goal of neuroscience research is to describe the dynamics of human cortical activity during cognitive tasks in real time. In this study, we employed stereo-electroencephalography (sEEG) – a high temporal resolution technique – in order to assess the neural activity of patients during tool action observation. We recorded the neural activity of 49 epileptic patients implanted with intracerebral electrodes, while they observed tool and hand actions. We assessed how different regions of the brain respond to the different events in the stimuli.

MAIN FINDINGS TO DATE

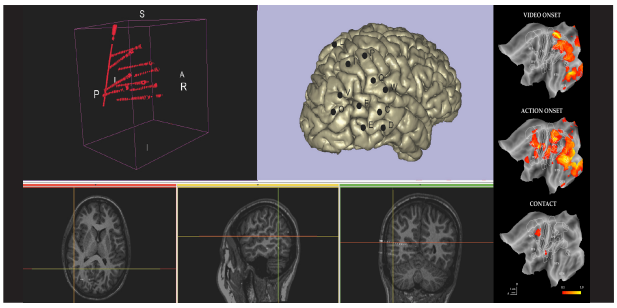
Observing tool action onset activated the action observation network but also, unlike hand action, a specific region in the left rostral inferior parietal lobule (aSMG). The final contact between the effector and the target object triggers activity in areas SII and premotor cortex.

SOCIO-ECONOMIC IMPACT

The absence of temporal resolution in imaging techniques has hindered the acquisition of four-dimensional maps of human neural activity. Here we show how monitoring the timing of cortical brain events during observation task allows for a better understanding of how the brain functions.

THE RESEARCH TEAM

This research team is led by Prof. Giacomo Rizzolatti, Dr Fausto Caruana and Dr Pietro Avanzini in collaboration with Prof. Giorgio Lo Russo, Dr Ivana Sartori, and Prof. Guy A. Orban affiliated with the Surgical Center for Epilepsy in Ospedale Niguarda, Milan and the Department of Neuroscience, University of Parma. Prof. Giacomo Rizzolatti is at the Department of Physiology and Biochemistry, University of Malta and at CNR Center of the University of Parma.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

This research is supported by the Cariparma Foundation and a European Research Grant (ERC).

TREATING EPILEPSY WITH LIGHT

OBJECTIVES OF THE RESEARCH

Epilepsy is a widespread neurological disease affecting 1% of the world population (about 2000 people in Malta). Many epilepsy patients are resistant to traditionally drug treatments or suffer from serious drug-induced side effects. Therefore, novel therapeutic approaches are desperately needed for controlling epilepsy and improving patients' quality of life. This project is investigating whether a novel technique, called optogenetics, can be used to treat epilepsy. Optogenetics uses light of different wavelengths to either stimulate or block nerve impulses of neurons that carry light-sensitive proteins.

MAIN FINDINGS TO DATE

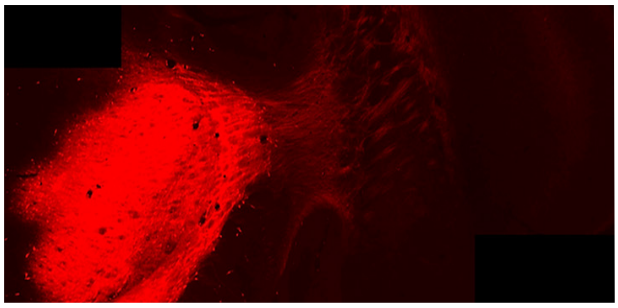
We have shown that a fibre optic implanted in the brain can deliver light pulses that block the abnormal activity of select neurons. We have developed a "closed-loop" system for controlling the flow of light impulses. Our prototype detects the brain electrical activity associated with an epileptic seizure within one tenth of a second. This "warning" signal is sent into the fibre optic and delivers a light pulse to the selected neurons. Preliminary results show that our prototype system is capable of stopping 100% of seizures without any external human intervention.

SOCIO-ECONOMIC IMPACT

Our research could result in an alternative treatment to brain surgery for epileptic patients who do not respond well to standard treatments with anti-epileptic drugs. Moreover, the new method could be useful for treating epileptic patients who have to stop taking anti-epileptic drugs due to adverse side-effects.

THE RESEARCH TEAM

The team consists of Prof. Vincenzo Crunelli in collaboration with Prof. Giuseppe Di Giovanni of the Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta), Dr Adrian Attard Trevisan (AAT Research Ltd., Malta) and Dr Magor Lorincz (University of Szeged, Hungary) and post-doctoral researchers Drs Gabriele Deidda, Francois David and Gergely Orban and PhD students Liad Baruchin, Maria Vella and Francis Delicata.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

This work is supported by a European Union research grant and MCST project grant (R&I-2013-014) "Closed-loop Serotonin Optogenetic Stimulation with EEG recording to Suppress Epileptic Seizures: A Therapeutic Device".

AUTISM SPECTRUM DISORDER WITH INTELLECTUAL DISABILITY

OBJECTIVES OF THE RESEARCH

The objectives of our research are to uncover disease causing mechanisms and potential therapies for several channelopathies. Recently, we uncovered a distinct ion channel-dependent disease characterized by ASD/ID, epilepsy and motor impairment. We are studying a large number of these patients, characterizing their phenotypes thoroughly and, analysing the presence of mutations in some inwardly-rectifying potassium channel types By using advanced electrophysiological, biochemical and confocal microscopy technologies, we will investigate the effects of mutations on channel activity, protein trafficking, calcium signals, astrocyte\neuron interaction and test drugs that are expected to ameliorate the symptoms.

MAIN FINDINGS TO DATE

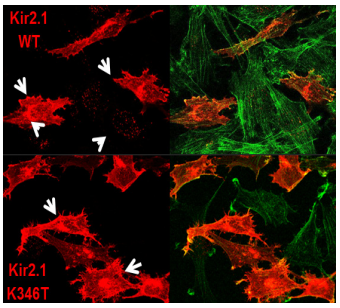
The genetic mutations in Kir2.1 and Kir4.1 channels and their molecular consequences have been partially clarified by our research team, which has provided new insight into the pathophysiology of both ASD/ID and epilepsy. By altering the channel properties, the identified mutations emerge as potentially deleterious for the functions of astrocytes and neurons in the brain.

SOCIO-ECONOMIC IMPACT

The outcome of our research is to transfer the knowledge and technology to clinical practice in order to advance diagnosis and improve cost-effectiveness. Families at risk will greatly benefit from our investigations as medical practitioners will help them preventing this channelopathy and sudden deaths, planning future pregnancies, predicting the clinical course of their affected child and getting the right therapy.

THE RESEARCH TEAM

The research team consists of Dr Maria Cristina D'Adamo (co-ordinator of the project) in collaboration with the University of Malta, and Dr Federico Sicca from University of Pisa and Dr Elena Ambrosini from the Istituto Superiore di Sanita, Rome. Scientists from Boston Children's Hospital of the Harvard Medical School have recently joined our team.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

This research programme is currently funded by the University of Malta and Fondazione Cassa di Risparmio di Perugia, Italy.

CURING DEPRESSION, ADDICTION AND EPILEPSY

OBJECTIVES OF THE RESEARCH

Brain diseases can affect anyone. One in three Maltese citizens and about 1 billion people worldwide suffer from some form of mental condition or disease. Depression, addiction and epilepsy, in particular, are amongst the most challenging public health issues in the 21st century. We are using electrophysiological behavioural and neurochemical approaches and animal models to study better understand the pathophysiology of these brain diseases with the aim of identifying new drug targets.

MAIN FINDINGS TO DATE

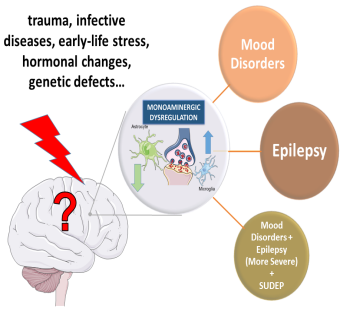
We have discovered one of the mechanisms involved in the antidepressant activity of the recreational drug Ketamine and that an area of the brain known as the Lateral Habenula is a potential new target for nicotine cessation therapies. In addition we found that marijuana, alcohol and cigarette smoking during childhood can induce long-term pathological brain changes as they become in adults. Lastly, we have discovered that increasing the levels of cannabinoids and serotonin can be used to treat epilepsy.

SOCIO-ECONOMIC IMPACT

Neuropsychiatric disorders have a high economic burden on society due to the debilitating health conditions. Although multiple therapies are currently available, most of patients are resistant to drug treatment. Our aim is to understand the direct neurobiological mechanisms involved in neuropsychiatric disorders, which will help us develop more efficacious treatments patients worldwide.

THE RESEARCH TEAM

The research projects are led by Prof. Giuseppe Di Giovanni of the University of Malta in collaboration with Dr Massimo Pierucci, Dr Roberto Colangeli, Dr Gergely Orban and Dr Gabriele Deidda and research students Francis Delicata, Maria Vella and Norbert Abela of the University of Malta; Prof. Vincenzo Crunelli and research exchange students of Cardiff University; Dr Maurizio Casarrubea, Prof. Arcangelo Benigno and Prof. Giuseppe Crescimanno of the University of Palermo and Dr Adrian Attard Trevisan of AAT Research Ltd.



ACKNOWLEDGMENT OF RESEARCH SUPPORT

The research is supported by the Malta Council for Science & Technology (R&I-2013-014), the University of Malta and the COST action CM1103. UK students are supported by ERASMUS plus.

MALTA LIFE SCIENCES PARK

Malta Life Sciences Park promotes research and development to spur the growth of the Life Sciences Sector in Malta. MLSP offers 8000 m^2 of offices and laboratories in a stimulating environment for knowledge-intensive companies. MLSP also supports and encourages commercial growth and success amongst its tenants by offering business and financial advice as well as assistance for technology development and internationalisation.

AAT

MENTE is a medical device developed to remove unwanted Delta and Theta brainwaves associated with autism. The device uses neurological data to create an inverse feedback signal using a state-of-the-art circuitry and noise-cancelling technology to suppress excessive Delta and Theta activity. This system is intended for children. The technology has been developed by Dr. Adrian Attard Trevisan and his team.

ELTY FOOD

A collaboration of highly experienced food specialists is establishing a database of properties of local food products which forms the basis of their intrinsic food research. They offer laboratory services including food chemistry analysis and pesticide residue level analysis. Elty Food helps the food producer with recipe development to ensure that his product is safe and healthy. The effort is being led by Ms. Jeanette Cameron.

MELISSA

A medical device this is being developed and commercialised that is clinically effective to treat wounds. The device is based on the healing properties of Maltese honey which has been clinically tested in Switzerland. Work now includes innovative methods to fine tune the formulation and ensure consistency of the device. The technology was developed by Mr. Ray Sciberras.

MALTA LABORATORIES NETWORK

The collaboration effort in the Network has seen the introduction of the Touch-DNA methodology where epithelia skin cells are used for DNA identification. This methodology is opening up a world of possibilities which links perpetrators' clothing to crime scenes. The Malta Police Crime Scene Investigation utilise the locally based accredited BioDNA Laboratory services. This technology implementation is being led by Dr. Marisa Cassar and Inspector Charlot Casha and their teams.

RESOLVE

A novel solvent recycling machine is being designed and developed for industry to recycle solvent waste. The project funded by MCST RTDI 2014-026T and led by Dr. Stefan Mohnani is intended to be energy efficient in order to make it commercially viable.

MLSP is a Malta Enterprise project that was partially funded by ERDF 199 and ERDF 331 (ERDF 2007-2013).

ENTERPRISING WATER SOLUTIONS

OUR MANDATE

The MCAST Water Research and Training Centre was established in 2013 to provide a national focal point for the research and skills related to the themes of potable water, grey water and sea water/coastal waters. The Centre works in close collaboration with MCAST's Centre for Agriculture and the Institutes of Applied Sciences and Engineering & Transport.

OUR VISION

Malta has the lowest water resources index and the highest water competition index in the whole of the Mediterranean basin. It is thus imperative that Malta be at the forefront of water technology and in the research and development of complex water systems. The MCAST Water Research and Training Centre focuses on water enterprise, aiming to embody the application of creative ideas and innovation to practical solutions in the fields of potable water and grey water. Research and innovation within this Centre focus on the themes of water systems control, water efficiency and innovation and water quality enhancement.

OUR ACHIEVEMENTS

Main research thrusts of the Centre are targeted at grey water micro-solutions, apparent water loss control and the descaling of water. As an example, an innovative grey water micro-system is being run by the Centre in collaboration with Alter Aqua, to study the effects of specifically treated drains water on the growth of plants in a green-roof setup. Recent results indicate that various indigenous plants can be grown in a micro-environment with this 'dirty' water. Another research initiative has focused on innovative magnetic technologies for the descaling of water within commercial treatment plants.

WATER PARTNERSHIPS

The water research is strongly supported by a number of EU and local funding grants such as the ERDF funding of the Centre's laboratories, lead partnership in a Horizon2020 project focusing on water utilization in agriculture, and local funding from MCST Fusion and the Global Water Partnership. The research is run by a team of MCAST lecturers, scientists, engineers and undergraduate students. A high level of collaboration exists with CIWEM, the IWA, CIHEAM, Cranfield University, the WSC, and SEWCU.

RESEARCH, INNOVATION AND DEVELOPMENT TRUST (RIDT)

OUR MANDATE

The Research Trust of the University of Malta (RIDT) was established in 2011 as a key component of Malta's efforts to bolster investment in research and development on a national level. RIDT was set up specifically to engage with the community and encourage its various sectors to embrace the emerging need of supporting research at the University of Malta.

OUR VISION

Malta's future, like any progressive nation's lies in the ability to innovate and discover new aspects of human development. Investing in Malta's future is akin to investing in each and every citizen's future. RIDT has embarked on a new path, where individuals, businesses and organisations dare to dream of a new way forward for Malta in a unique partnership across public, private and social sectors.

OUR ACHIEVEMENTS

In the few years since its inception, RIDT has attracted broad ranging support from a number of sectors, thereby providing a tangible and much needed impetus for high-calibre R&D projects across various faculties and departments within the University of Malta, be it projects in engineering and technology, physical and life sciences, medicine, humanities, social sciences and the arts.

The Maltese community has responded to our appeal by financing a number of research initiatives including PhD scholarships in cancer research, a programme in kidney research, a nation-wide survey on diabetes, industrial research, studies in economics, osteoporosis, digital marketing, ICT and linguistics.

See more on our website: www.ridt.eu

THE UNIVERSITY OF MALTA MOBILE DENTAL CLINIC

This novel community project reaches out to all sectors of society including the underprivileged, the institutionalised, schools, orphanages and the homebound. While providing oral health advice and dental care, the project collects epidemiological data relating to the Maltese population. This project has been achieved thanks to the support of a number of corporate entities through RIDT.

MALTA MEDICINES AUTHORITY

OUR MANDATE

The Medicines Authority is a science and health oriented competent authority based on the values of quality, innovation, people and integrity. Our mission is to protect and enhance public health through the regulation of medicinal products and pharmaceutical activities.

OUR VISION

Our vision is to be a centre of excellence in advancing effective and innovative regulation and promoting quality and scientific rigour in the work we do. We strive to be a best in class regulator for the benefit of patients and stakeholders. We endeavour to be an internationally recognised, efficient entity and promoter of people development and sustainable growth.

ACHIEVEMENTS

Safe, Efficacious and Quality Medicines for the Benefit of the Patients

- Over 5300 medicinal products registered, with over 450 products having been registered between January and August 2016.
- Top 5 position in the EU in the assessment of generic medicines at centralised level and assessing over 250 medicinal products as Reference Member State.
- Establishment of Medicines Intelligence and Access Unit.

Effective and Innovative Regulation

- Conducting EU Good Manufacturing Practice Inspections in India, China, Serbia and Ukraine, establishing Malta as a hub for the pharmaceutical and life sciences sectors in Europe.
- First agency to deliver joint scientific advice with another Member State so as to facilitate accelerated access to medicines.
- Collaboration with the MEB of the Netherlands to start outsourcing part of its assessment work to Malta.

Achieving Results through People, Good Governance and Innovation

- Introduction of the Medicines Authority International Traineeship Programme and over 30% of employees carrying out studies at Masters and Doctoral level.
- An established and certified Quality Management System.
- Relocation to the strategic and scientific environment of the Malta Life Sciences Park leading to cost savings.

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