Comparative Genomics of *Brassica oleracea*.

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CONTENTS

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Carol Ryder

Declaration

The work presented in this covering document is the candidate's own work. Where the research was carried out collaboratively, this is stated in the text and the published work presented contains the names of the collaborators. Statements about the proportion of the work that was carried out by the AUTHOR are supplied by the collaborators where possible (Appendix 5.1 & 5.2, p50-64). Detailed descriptions of the AUTHOR'S contribution to each multi-author paper are provided for each publication (2.1 p13-14, 2.2 p18-20, 2.3 p24-25, 2.4 p29-31 and 2.5 p34-35).

The submitted material as a whole is not substantially the same as published or unpublished material that the AUTHOR has submitted for a degree, diploma, or similar qualification at any university or similar institution.

Signed

Carol Diana Ryder

Abstract

The scientific case made by the AUTHOR'S comparative *Brassica oleracea* genomics work is presented through 5 peer reviewed research papers. In order to achieve a comprehensive understanding of the evolution of *B. oleracea* the identification of unique genome characteristics, established using comparative genomics, is required. The genome characteristics established within these papers deliver significant contributions to original knowledge. These include a detailed illustration of how macro scale synteny varies markedly between the *B. oleracea* and *A. thaliana* genomes; unambiguous integration of the *B. oleracea* cytogenetic and genetic linkage maps; a cross species characterisation of a large collinear inverted segmental duplication on a single *B. oleracea* chromosome establishing that the relative physical distances have stayed approximately the same; retrotransposon copy number estimations and characterisation of their genomic organisation and isolation, characterisation and cross species analysis of a C genome specific repeat. For each paper the AUTHOR'S individual scientific contribution to each aspect of the work is described in detail. Both individually and as a body of work these publications substantially advance the fields of comparative, Brassica and genomic research.

Table of Abbreviations and Acronyms

TABLE 1: Table of Abbreviations and Acronyms

Acronym	Meaning
AUTHOR	The author of this document.
A&M	Texas 'A&M' University
BAC	Bacterial Artificial Chromosome
BBSRC	Biotechnology and Biological Sciences Research Council
BoB	*B. oleracea* BAC
CPRO-DLO	Centre for Plant Breeding and Reproduction Research-Agriculture Research Department
EST	Expressed Sequence Tag
HRI	Horticulture Research International
IGF	Investigating Gene Function
FISH	Fluorescence *In Situ* Hybridisation
LARS	Long Ashton Research Station
MBGP	Multinational Brassica Genome Project
Mya	Million years ago
NCBI	National Centre for Biotechnology Information
PI	Principal investigator
RFLP	Restriction Fragment Length Polymorphism
TE	Transposable Element
TIGR	The Institute for Genomic Research
WHRI	Warwick – Horticulture Research International

1. Introduction

This document presents the scientific basis for AUTHOR'S research and precisely explains the AUTHOR'S role, both intellectually and technically. The field of comparative Brassica genomics is briefly defined which sets the scene for the direction of this body of work. Chapter 2 presents each publication sequentially and summarises its scientific content, significance and contribution to knowledge. Subsequent scientific advances enabled by each publication are also presented. The AUTHOR'S individual contribution to each multi-author paper is clearly set out. The interrelationship between the materials presented is described and the conditions and circumstances under which each piece of work was carried out are outlined. Chapter 3 draws this work to a conclusion by explaining how, both individually and as a body of work, these publications substantially advance the fields of comparative, Brassica and genomic research. The appendix (section 5) contains a percentage contribution breakdown for each presented paper; statements from co-authors corroborating the AUTHOR'S intellectual, technical and written contribution to each manuscript; a summary of the AUTHOR'S research career; a full bibliography of the AUTHOR'S published work and its associated citation information.

The AUTHOR has 15 plant genetics and genomics publications in peer reviewed journals which address a broad range of plant genetics and genomics questions. Both the *Brassica* and rosaceous species have been researched and a common theme throughout has been the use of comparative genomics to study plant evolution. From this broad body of work the AUTHOR selected five highlights that show this common theme for *B.*

oleracea. However, the publications not selected for presentation serve to provide additional evidence of the AUTHOR'S research background and contribution to plant genomics.

The field of comparative Brassica genomics

Comparative genomics is the study of genomic relationships between different species. The steady accumulation of genomic resources (e.g. whole-genome sequences, expressed sequence tag [EST] libraries, high throughput resequencing technologies) over last 20 years has allowed comparative genomics to be used as a tool to address diverse biological research questions.

Arabidopsis thaliana was officially designated as the model plant species in 1998 (Fink, 1998) although research on this rapid cycling flowering plant with a relatively small genome extends back over a century.

Phylogenetic approaches reveal that Arabidopsis and Brassica lineages diverged 37-51 million years ago followed by a Brassica whole-genome triplication event 22.5 Mya (Beilstein *et al* 2010). The phenotypically diverse species *B. oleracea* is widely considered to be one of the closest crop relatives to the model plant species *A. thaliana* (Beilstein *et al* 2010) and thus is an ideal research subject. **Thesis statement: This study seeks to show how a comprehensive understanding of the evolution of *B. oleracea* requires the identification of unique genome characteristics established using comparative genomics.**

The Brassicas make up 39 species of the tribe Brassiceae within the plant family Brassicaceae (or Cruciferae). The family is comprised of c.340 genera

and over 3000 species (Warwick *et al* 2009). Species of the *Brassica* genus are of agricultural importance as oil seeds, vegetables and fodder crops. Examples of these are *B. napus* (oil seed rape, canola), *B. rapa* (chinese cabbage, turnip), *B. juncea* (mustard) and *B. oleracea* (cabbage, broccoli, Brussels sprouts, cauliflower, kohlrabi and kale). The close relationship between 6 particularly important species is described by the theory 'Triangle of U' (U, 1935). This theory explains the evolution and relationships between the 6 species and suggests that the genomes of three diploid ancestral *Brassica* species (*B. rapa* (AA), *B. nigra* (BB) & *B. oleracea* (CC)) combined to create three tetraploid Brassica species (*B. carinata* (BBCC), *B. juncea* (AABB) & *B. napus* (AACC)). Because the C genome is a cornerstone in the triangle of U and thus an integral part of *B. napus* and *B. carinata* genomes, *B. oleracea* research facilitates the wider field of Brassica genome research. Of wider significance, understanding the evolutionary relationships between this set of related genomes has the potential to inform studies in other species, particularly those with polyploid genomes.

2. Contributions to the Field of Comparative Brassica Genomics

A summary of the interrelationships between the presented work and the scientific advances they have facilitated is depicted in Figure 1. This serves as a visual aide for the reader.

Interrelationships between presented work and its scientific impact

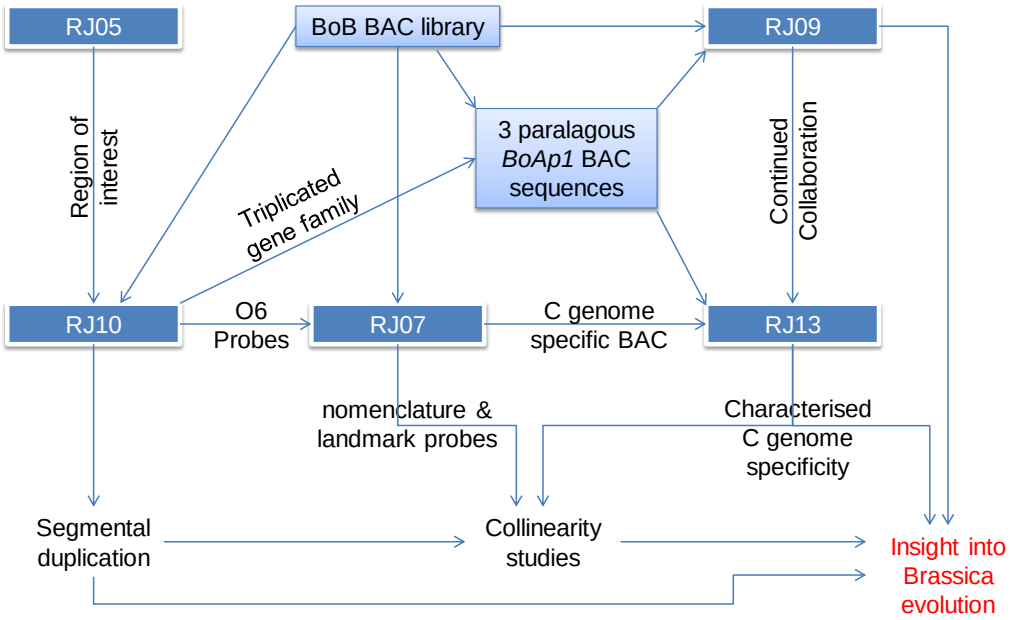

Figure 1 - Interrelationships between presented papers and the scientific case they make. The AUTHOR'S published work is referenced in the style (RJ##). This style of referencing prevails throughout this manuscript.

2.1 (RJ05) Contrasting genome organisation: Two regions of the *Brassica oleracea* genome compared with collinear regions of the *Arabidopsis thaliana* genome.[1]

Hypothesis, scientific approach and conclusions

This paper tested the hypothesis that collinearity between the *B. oleracea* and *A. thaliana* genomes varies significantly depending on the region analysed. It investigated the organisation and relationship between two defined and contrasting regions of the *B. oleracea* genome and the organisation of corresponding sequences in *A. thaliana*. This was achieved by hybridising genetically mapped *B. oleracea* low copy number DNA fragments onto the whole *A. thaliana* genome in the form of macro arrayed filters of physically mapped BAC clones.

Our approach contrasted with that of previous Cruciferae collinearity studies. Lan *et al* (2000) had established a detailed comparative map of *B. oleracea* and *A. thaliana* based largely on RFLP mapping of *Arabidopsis* ESTs both in *Arabidopsis* and *Brassica* populations. Rather than relying on genetic mapping, our work utilised the complete Arabidopsis physical map and placed specific regions of the *B. oleracea* genetic map in the context of *A. thaliana*. Using a solely physical mapping approach O'Neill and Bancroft (2000) had also analysed aspects of gene conservation and microsynteny using probes from a 222kb region of the *A. thaliana* genome to construct *B. oleracea* BAC clone contig maps to analyse the gene content and organisation of a set of paralogous segments. Although a high level of detail can be revealed by this approach its application to larger regions is limited

[1] C.D. Ryder, L.B. Smith, G.R. Teakle & G.J. King. (2001) Genome. 44: pp808-817

not only due to the high costs involved but also due to its reliance on conserved synteny, which as the authors' work demonstrates, is frequently absent. At the time this work was carried out the *A. thaliana* genome sequence was incomplete, hence the physical map was the most comprehensive resource available for investigating cross species collinearity. The physical map of the *A. thaliana* genome was established in the late 1990's (Mozo *et al* 1999). By using macro-arrayed colony filters of the BAC genomic libraries from which the physical map was constructed the AUTHOR was able to physically locate and quantify *A. thaliana* loci homologous to the *B. oleracea* floral regulatory gene families *BoCAL* and *BoAP1* prior to the release of the *A. thaliana* sequence. These floral genes were central to the work of colleagues (Smith & King, 2000) and guided the selection of regions for this study. Additional hybridisations using neighbouring markers from the *B. oleracea* genetic map allowed collinearity to be investigated. The complete *A. thaliana* genome sequence of all five nuclear chromosomes was first published in 2000 (The Arabidopsis Genome Initiative). This has been frequently updated, corrected and annotated in the subsequent years. Version 10 was current at the time of writing (2012) (http://www.arabidopsis.org). Early analysis of this sequence showed extensive duplication within the model plant genome (The Arabidopsis Genome Initiative, 2000 & Blanc *et al* 2000). Albeit with different date estimates, the hypotheses that Arabidopsis and Brassica lineages shared a common ancestor and that Brassica subsequently underwent a whole-genome triplication event were documented at the time this work was carried out (Lagercrantz & Lydiate 1996, Yang *et al* 1999, Koch *et al* 2000). These

findings all complicate the concept of inferring information from one genome to another. We were aware of these complications whilst conducting our research and hence focused on how to use comparative genomic analysis to help solve this complex problem.

This paper validated the hypothesis that collinearity varies significantly depending on the region analysed. It revealed that the top section of linkage group O6 and all of the selected section of linkage group O3 displayed considerable evidence of rearrangements with respect to *A. thaliana*. In contrast, an intriguing pattern of inverted segmental duplication with respect to *A. thaliana* was revealed on linkage group O6.

Scientific advances enabled

This paper is widely cited as an illustration of the variable collinearity between Arabidopsis and *B. oleracea*: (e.g. Gao *et al* 2003, Lukens *et al* 2003, Cogan *et al* 2004, Ayele *et al* 2005, Walley *et al* 2012). Furthermore, because the *B. oleracea* C genome is conserved within the amphidiploid *B. napus* this study has also informed *B. napus* collinearity studies (Osborn *et al* 2003, Wang *et al* 2011). Research interest has been shown in the possibility that the self-incompatibility (*S*-) locus locates close to the junction of the two inverted and duplicated segments and the potential breeding implications this may have. Knowledge of the *B. oleracea* genome regions investigated by this paper is now greatly improved although its findings are nevertheless consistent.

This initial characterisation of collinearity forms the basis for a more detailed study presented in RJ10 and thus assisted in the physical and genetic map integration presented in RJ07. Furthermore, as a result of the relationship

revealed, the *BoAP1* gene family (*BoAP1-a* (region A) and *BoAP1-c* (region B)) within linkage group O6 inverted segmental duplication was selected as a case study for syntenic relationships at a more detailed level. Along with the third member of the gene family, *BoAp1-b* mapped to linkage group O2 (not in this paper) a representative BAC clone was sequenced for each locus. The sequences from these collinear paralogous loci are utilised in RJ09 and RJ13. Finally, the illustration of variable collinearity and the inferred inverted segmental duplication add to a repertoire of established genome characteristics required for corroboration of *B. oleracea* whole genome sequence assembly. Analysis of whole genome sequence comparisons will allow a comprehensive understanding of *B. oleracea* evolution to be attained.

AUTHOR'S contribution and working environment

The hypothesis of evaluating cross species genome collinearity was conceived at a group meeting and hence developed in a team discussion environment with all authors contributing. This team discussion also led to the selection of regions for investigation. The working strategy, scope and methodology for this work were developed by the AUTHOR and reviewed by the team leader (Dr King) to ensure they could be supported in light of the wider departmental resources and work load. The AUTHOR carried out all practical work, data analysis and interpretation contained in this manuscript with the exception of re-calculating an updated version of an integrated genetic map enabling data to be presented in the context of the most recent data available at the time. The AUTHOR created all tables and figures, and wrote the majority of the text. The style and content of the document was greatly enhanced by the input of Dr King.

This work was sponsored by a BBSRC core strategic grant (CSG), all authors were members of the same research team and the AUTHOR was employed as a research scientist.

2.2 (RJ07) Integration of a cytogenetic map with a genetic linkage map of *Brassica oleracea*.[2]

Hypothesis, scientific approach and conclusions

We hypothesised that we could link karyotype to a genetic linkage map using marker tagged low copy FISH probes. By unambiguously pairing each of the nine *B. oleracea* chromosomes to its corresponding linkage group using a good quality genetic map this paper sought to establish a reliable karyotype with chromosome-specific landmarks to identify and orientate each chromosome relative to the genetic map. In doing so we aimed to facilitate all future comparative genomic studies involving *B. oleracea* and the Brassica C genome in other contexts *(B. napus, B. carinata)*. In 2002 there had been numerous *B. oleracea* genetic maps established (Bohuon *et al* 1996; Sebastian *et al* 2000; Camargo *et al* 1997; Kianian *et al* 1992; Hu *et al* 1998; Lan & Paterson 2001) and the *B. oleracea* chromosomes had been studied cytogenetically (Armstrong, 1998). However the two had not been placed into context with each other.

The FISH technique was used by colleagues at the University of Birmingham to probe meiotic metaphase spreads with carefully selected DNA fragments representing genetically mapped markers. Due to the replicated and repetitive nature of the *B. oleracea* genome many candidate genetic loci failed to yield suitable FISH probes. Conversely some candidate FISH probes behaved unexpectedly when hybridised to whole chromosomes. Each BAC/clone placed on the cytogenetic map is the result of a painstaking

[2] Howell, EC, Barker, GC, Jones, GH, Kearsey, MJ, King, GJ, Kop, EP, Ryder, CD, Teakle, GR, Vicente, JG, Armstrong, SJ. (2002) Genetics 161(3): 1225-1234

detective story using available data and diverse molecular biology techniques as required to reach an unambiguous conclusion.

The BACs used in this paper were all from a library made by the AUTHOR whilst on secondment to Texas A&M University crop biotechnology centre in 1997 (Vicente & King, 2001; RJ09). This was the first Brassica BAC library ever to be constructed and its clone names are prefixed by 'BoB'.

This paper presents all nine linkage groups of a *B. oleracea* genetic map assigned to the corresponding chromosome of the parental karyotype. It orientates eight of the nine pairings and suggests the most probable orientation for the ninth.

Scientific advances enabled

This integration underpins all subsequent work on the Brassica C genome and has facilitated the addition of markers to the C-genome chromosomes (Geleta *et al* 2012). Traditionally, chromosomes in cytogenetic maps are numbered in size order with the longest being number 1 (Armstrong, 1998). At the time this paper was written no linkage group numbering convention existed for *B. oleracea* genetic maps. Thus, 'linkage group 1' in one genetic map may not represent the same chromosome as 'linkage group 1' in another map (e.g. Hu *et al* 1998). The act of pairing linkage groups to chromosomes initially resulted in an added layer of complexity to this already multifaceted nomenclature system. For many years, when cross referencing between maps, it was necessary to be specific about which genetic map was being discussed, which linkage group, it's orientation and corresponding chromosome number. Consequently, in 2007 the MBGP Steering Committee agreed a consistent, *B. napus* centred, chromosome/linkage group

nomenclature for diploid *Brassica* genomes in the 'triangle of U' http://www.brassica.info/resource/maps/lg-assignments.php. The linkage group nomenclature presented in RJ07 (2002) conforms entirely to this system, thus reinforcing its position as a definitive text for *B. oleracea* genome characterisation. The agreed nomenclature associates a physical chromosome with a genetic linkage group and assigns the pair a single designation (e.g. see RJ10, published prior to the MBGP agreement).

This work is widely cited as an example of successful integration of cytological and genetic maps (e.g. Wang *et al* 2006, Lim *et al* 2006 & Figueroa *et al* 2012). As with RJ05 our characterisation of the *B. oleracea* 'C genome' has informed *B. napus* research (e.g. Osborn *et al* 2003, Udall *et al* 2005, Parkin *et al* 2005 and Xiong & Pires, 2011). It has also facilitated numerous collinearity studies (e.g. Parkin *et al* 2005 & Ziolkowski *et al* 2006).

The BoB library used in this paper is recognised within the Brassica research community as a high quality research resource and as such was used in projects such as the BBSRC funded 'Brassica IGF project'. This sought to construct a physical map of the *B. oleracea* genome using macro arrayed BAC library screening with single locus *A. thaliana* probes in combination with BAC fingerprinting (http://brassica.bbsrc.ac.uk/IGF/). This approach yielded a multitude of BAC contigs for *B. oleracea*, although the highly repetitive nature of the genome prevented a cohesive physical map being produced. Because this paper anchors some BoB BACs to physical chromosomes, and some of these BACs are in IGF contigs, it therefore suggests a location for these contigs. Numerous international research teams are using the landmarks identified by this paper to facilitate genome

navigation, whilst adding their own BAC clones and hence mapping their genes of interest, e.g. Irwin *et al* 2012. Subsequent to publication a BAC reported in this paper (BoB014O06 (Chr5 LGO2)) was used without $C_o t$-1 DNA (to block repetitive sequences), and found to hybridise selectively to Brassica C genome and not A genome chromosomes. This discovery has been used extensively within the Brassica research community (e.g. RJ11, RJ12, Mason *et al* 2010, Szadkowski *et al* 2010, Ge *et al* 2009 and Nicolas *et al* 2008 & 2009). The CACTA transposon characterised in RJ13 is almost certainly causative of this observed C genome specificity. Finally, linking karyotype to a linkage map and discovering unique probes which enable genome navigation add to a repertoire of established genome characteristics required for corroboration of *B. oleracea* whole genome sequence assembly. Analysis of whole genome sequence comparisons will allow a comprehensive understanding of *B. oleracea* evolution to be attained.

AUTHOR'S contribution and working environment

The hypothesis of linking karyotype to a genetic linkage map using marker tagged low copy FISH probes was developed collaboratively with the AUTHOR, Elaine Howell, Susan Armstrong and Graham King contributing equally. The AUTHOR had significant input into the grant proposal that led to this work taking place. The AUTHOR took sole responsibility for identifying candidate probes with which the FISH analysis was performed by Elaine Howell. It was originally envisaged that mapped RFLP clones would serve directly as FISH probes however the small size (<3kb) of these clones meant that their fluorescence signal was rarely detectable. An alternative working strategy was developed by the AUTHOR and Elaine Howell using larger (50-

200kb) clones from the AUTHORS BoB BAC library as FISH probes. In the majority of instances this amended working strategy dictated that identification of suitable probes required genetic marker development to achieve position confirmation on the linkage map. The AUTHOR designed all required genetic markers.

Due to the replicated and repetitive nature of the *B. oleracea* genome and the consequential unpredicted behaviour of many candidate unique landmark probes the project necessitated frequent detailed dialogue to achieve unequivocally reasoned conclusions, and to decide future strategy. The bulk of this dialogue and execution of all laboratory work was done by the AUTHOR and Elaine Howell (the paper's lead author). Joana Vicente and Erik Kop identified BACs putatively associated with their mapped marker assays, Graham Teakle calculated genetic map positions for the new markers and Guy Barker provided knowledge of BAC contigs from the IGF physical map project. Compilation of the manuscript was a team effort necessitated by the deductions for each landmark requiring detailed, intricate yet unambiguous explanation. The AUTHOR, Elaine Howell, Susan Armstrong and Graham King were the major contributors to manuscript preparation. In addition to sections of text the AUTHOR prepared Table 1 and had a large input into Fig. 2.

In contrast to current convention regarding author order this multi-author paper lists the authors in alphabetical order, with the exception of first author (first draft and manuscript submission) and last author (corresponding author). Had current convention been adopted the AUTHOR would have been placed second.

This collaboration with Birmingham University was funded by a 3yr BBSRC grant on which the AUTHOR was a named researcher employed as a research scientist. This same grant also funded the work in RJ10.

2.3 (RJ10) Physical organisation of the major duplication on *Brassica oleracea* Chromosome O6 revealed through fluorescence *in situ* hybridization with *Arabidopsis* and *Brassica* BAC probes.[3]

Hypothesis, scientific approach and conclusions

In this paper we test the hypothesis that each segment of an inverted duplication on *B. oleracea* O6 displayed significant collinearity with a 5.2 Mb segment of *A. thaliana* chromosome 1 and also that the relative physical distances between the markers in the two segments of O6 and *A. thaliana* had stayed approximately the same. This piece of work aimed to confirm the presence and increase the local resolution of the inverted segmental duplication covering the majority of the long arm of a short *B. oleracea* chromosome, O6 region inferred by RJ05.

This was achieved using *B. oleracea* and *A. thaliana* BAC probes for FISH analysis of *B. oleracea* pachytene chromosomes. Since meiotic pachytene chromosomes are less condensed than the mitotic metaphase chromosomes used in RJ07, we were able to increase the resolution markedly. We examined collinearity of marker order by sequentially probing with pairs of clones. Each clone was separately labelled either red or green hence their signal location(s) could be observed relative to each other and to the telomere. We measured all test probes relative to a consistent reference BAC and to the telomere to allow for the changes in chromosome

[3] E.C. Howell, G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, C.D. Ryder, S.J. Armstrong. (2005) Genome. 48(6): pp1093-1103.

condensation during meiosis, these measurements allowed us to assess physical distance relationships.

At the time this work was carried out whole genome collinearity studies of *B. oleracea* and *A. thaliana* had been published using sequenced, genetically mapped *B. oleracea* markers and the complete *A. thaliana* sequence (Lan *et al* 2000; Li *et al* 2003; Lukens *et al* 2003). The methodology used in these studies was unable to assess physical distance relationships between the two genomes. They all gave insights into the duplicated/triplicated nature of the *B. oleracea* genome relative to *A. thaliana* but all conclude that much higher density genetic or physical maps are required. Collinearity had also been studied by comparing *B. oleracea* whole BAC sequences to the *A. thaliana* sequence (Quiros *et al* 2001; Gao *et al* 2004; O'Neill and Bancroft 2000). In essence these studies showed a conserved gene order with gene insertions, deletions and duplications commonly observed. Whilst these studies do facilitate physical distance comparisons between the two genomes the largest *A. thaliana* region analysed was 222kb.

Our approach achieved a 23-fold increase in size of region analysed. We compared a 5.2 Mb segment of *A. thaliana* to homoeologous regions of the *B. oleracea* genome and also compared paralogous *B. oleracea* segments. We increased the local resolution of the *B. oleracea* cytogenetic map and characterised segments of common origin in *Arabidopsis* and *B. oleracea*. Combining evidence from genetic and cytogenetic maps enabled us to characterise the relationship between genetic (recombination) and physical chromosome distances over a defined region of a *B. oleracea* chromosome. We demonstrated that the physical marker order was well conserved

between the two genomes and showed that the relative physical distances of markers in the more distal segment of *B. oleracea* and *A. thaliana* have stayed approximately the same. The more proximal (centromeric) *B. oleracea* segment proved a challenge to analyse because fewer measurements were possible. The synizetic knot (a coalescence of pericentromeric heterochromatin) observed at the pachytene stage of meiosis frequently obscured this section of chromosome. This resulted in larger confidence intervals but suggested that this segment was largely consistent in both marker order and physical size. We demonstrated that FISH analysis using BAC probes from a sequenced model genome was highly effective in establishing the relative size and arrangement of regions within related but more complex genomes.

Scientific advances enabled

Our methodology has been adapted for use in cereal genomics using *Brachypodium distachyon* as the model species for temperate cereals and grasses (Jenkins & Hasterok, 2007). This work is widely cited as an example of FISH use in comparative plant genome research (e.g. Jiang & Bikram, 2006, Iovene *et al* 2008, Koo & Jiang, 2009, Lou *et al* 2010) and as a methodology for achieving integration of genetic, physical and cytogenetic maps (Xiong *et al* 2010, Han *et al* 2011). The AUTHOR is aware of unpublished research which is using this approach to complement and inform the construction of BAC physical contigs within complex genomes.

This study adds to the steadily accumulating knowledge of syntenic relationships between the *B. oleracea* and *A. thaliana* genomes (Wang *et al* 2011) and facilitated further comparative genomic studies in Brassica (e.g.

Ziolkowski *et al* 2006). It is considered a milestone in comparative evolutionary Brassicaceae genomics by Lysak & Lexer (2006). The *B. oleracea* BACs established as landmark probes in this study have subsequently been used simultaneously to navigate the C6 and collinear A7 chromosome of the *B. napus* genome (Howell *et al* 2008). Similarly, orthologues of the *FLOWERING LOCUS T* (*FT*) gene have been found to locate within *B. napus* inverted duplicated regions of chromosomes A7 and C6 (Wang *et al* 2009) illustrating the conserved nature of the polyploid genome. Finally, the characterisation of this region of C6 adds to a repertoire of established genome characteristics required for corroboration of *B. oleracea* whole genome sequence assembly. Analysis of whole genome sequence comparisons will allow a comprehensive understanding of *B. oleracea* evolution to be attained.

AUTHOR'S contribution and working environment

The hypothesis of a collinear segmentally inverted duplicated repeat with conserved relative physical distances was developed through discussion between the AUTHOR and Graham King. After developing the hypothesis Elaine Howell, Susan Armstrong and Mike Kearsey suggested the use of multicolour cross species FISH at the pachytene stage of meiosis. The AUTHOR had significant input into the grant proposal that led to this work taking place.

The AUTHOR took sole responsibility for providing candidate probes with which to perform FISH analysis whilst Elaine Howell carried out all FISH work. There was frequent dialogue between the AUTHOR and the paper's

lead author, thus ensuring robust, unambiguous conclusions were reached. Working relationships were similar to those explained for RJ07.

Guy Barker provided knowledge of BAC contigs from the IGF physical map project. Compilation of the manuscript was an iterative process of embellishment following the first draft. The manuscript was passed between the AUTHOR, Elaine Howell, Mike Kearsey and Graham King before being verified by the other three co-authors. In addition to sections of text the AUTHOR prepared Table 1 and had a large input into Figs. 1 and 3.

In contrast to current convention regarding author order this multi-author paper lists the authors in alphabetical order, with the exception of first author (first draft and manuscript submission) and last author (corresponding author). Had current convention been adopted the AUTHOR would have been placed second.

This collaboration with Birmingham University was funded by a 3yr BBSRC grant on which the AUTHOR was a named researcher employed as a 'post-doctoral' research scientist (research fellow). This same grant also funded the work in RJ07.

2.4 (RJ09) The genomic organisation of retrotransposons in *Brassica oleracea*.[4]

Hypothesis, scientific approach and conclusions

We hypothesised that the highly replicated fraction of the *B. oleracea* genome was not homogenously organised. The study sought to expand on existing comparative surveys which had almost invariably focused upon low copy number anchored markers and/or sequenced expressed coding regions. By studying the organisation and distribution of repetitive sequences we aimed to facilitate comparative genomic studies and consequently provide a more comprehensive understanding of the genome evolution of the species since divergence from the common ancestor of the Brassicaceae. Our approach would also provide insights into the organisation of retrotransposons with respect to genes and achieve additional physical mapping of sequences in the genome.

The vast majority of sequences conserved between plant genomes are transposable elements (TEs) which make up the largest fraction of the genomes of most multicellular organisms. This fraction of the genome is frequently overlooked by comparative genomic studies, especially where complete genome sequence is not available, as remains the case for *B. oleracea*. Because these repetitive sequences significantly hinder long range sequence assembly, a genome with a high TE fraction is less likely to have a complete genome sequence available. Whilst TEs are often considered 'junk DNA' they can significantly alter the genome size of an organism, they can

[4] K. Alix, C.D. Ryder, G.J. King, J. Moore & J.S. Heslop-Harrison. (2005) Plant Molecular Biology. 59(6): pp839-851

create phenotypically significant mutations and are prevalent in the *B. oleracea* genome. Transposons are assigned to one of two classes according to their mechanism of transposition, which can be described as either "copy and paste" (Class I) or "cut and paste" (Class II).

This paper looked at five representative Class I TEs (retrotransposons) in *B. oleracea* and analysed their copy number and genomic organisation. This was achieved using a combination of genomic library homology screening, FISH and bioinformatics sequence analysis.

Prior to our study a non-random distribution of TEs in the *A. thaliana* genome had been observed by Peterson-Burch et al (2004). Whole genome shotgun sequences of *B. oleracea* covering 283 Mb (0.44×) of the estimated 650 Mb genome had been made publicly available by TIGR in the Genome Survey Sequence (GSS) division of GenBank (Ayele *et al* 2005). Zhang and Wessler (2004) had compared TEs in the *A. thaliana* sequence with those in the GSS of the much larger *B. oleracea* genome to estimate the patterns of amplification, diversification and loss since the species diverged from a common ancestor. They found nearly all TE lineages were shared, and conclude that both species inherited and retained largely the same collection of TEs from their common ancestor and amplification of TEs contributed largely to the difference in genome size. They estimated the total length of TEs in *B. oleracea* to be ~120 Mb or 20% of its genome (class I elements ~78 Mb, 14%; class II elements ~37 Mb, 6%). This was ~15 times more than the 8 Mb, 6% found in *A. thaliana*.

By focusing on the organisation of retroelements our work built on these existing studies. We investigated genome distribution of 5 selected

retrotransposons using FISH and showed that each retroelement had a characteristic genomic distribution. We estimated copy number of the same 5 elements using gridded ‘‘BoB’’ BAC library hybridisations and revealed that four individual LTR retrotransposons were represented by between 90 and 320 copies in the haploid genome whilst only a single location for a LINE was estimated. Sequence analysis of the same elements against GSS gave estimates of between 60 and 570, but no LINE was found. We showed minimal evidence for clustering between any of these retroelements as only half the randomly expected number of BACs hybridized to both LTR-retrotransposon families. Seven BAC sequences were analysed for their gene and TE content and revealed marked differences in overall TE numbers in each BAC. Estimations of TE density per BAC range from 1 every 82kb to 1 every 6kb. Our results suggest there are preferential sites and perhaps control mechanisms for the insertion or excision of different retrotransposon groups.

Scientific advances enabled

Our report that FISH hybridization signal patterns for Bo10COP-18 concentrate in pericentromeric regions of *B. oleracea* chromosomes has been utilised to efficiently develop PCR based polymorphic markers preferentially targeting centromeres (Pouilly *et al* 2008). Also, subsequent to the publication of our work Lu *et al* (2006) also found a high copy number of copia-like retrotransposons in the *B. oleracea* genome and went on to characterize the insertion of a copia-like LTR retrotransposon in their gene of interest. They found that this insertion resulted in the production of three alternatively spliced transcripts. The year after our work was published Hong

et al (2006) studied *B. rapa* transposable elements and estimated that TEs comprised 14% of the genome, with 12.3% class I elements. Investigations subsequently carried out in other species cite our study as a working reference e.g.; non-photosynthetic flowering plants (Park *et al* 2007); *Hypochaeris* (Asteraceae) (Ruas *et al* 2008); *Zizania latifolia* (wild rice) *(*Zhong *et al* 2009) *Triticum aestivum* (common wheat) (Ragupathy *et al* 2010). In addition, this work was cited as a noteworthy contribution to cytogenetics and genome analysis of Brassica crops by Snowdon, 2007. Finally, our characterisation of repeat distribution adds to a repertoire of established genome characteristics required for corroboration of *B. oleracea* whole genome sequence assembly. Analysis of whole genome sequence comparisons will allow a comprehensive understanding of *B. oleracea* evolution to be attained.

AUTHOR'S contribution and working environment

Discussions between the AUTHOR, Karine Alix, Pat Heslop-Harrison and Graham King resulted in the formulation of the hypothesis that the non-coding fraction of the *B. oleracea* genome was not homogenously organised. Initially our working strategy constituted a BAC library screen to estimate copy number and coincidence of hits complemented by FISH imaging to reveal the distribution of each selected TE. The AUTHOR subsequently instigated the addition of copy number estimates using genome survey sequence and the analysis of TE content/gene clustering using available whole BAC sequences into the working strategy.

In terms of execution of work the AUTHOR'S contribution involved construction and manipulation of the BAC library, advising and guiding with macro array

hybridisation and scoring of results, querying accumulated 'BoB' characterisation data from many research projects in search of coincidence of hybridisation trends, sequencing of 3 BAC clones, whole BAC sequence analysis of 7 BAC clones and southern blotting for the study of the LINE clone. The AUTHOR was actively involved in the design of experiments, interpretation of each set of results and discussions as to how they interacted to formulate a coherent argument.

Compilation of the manuscript was a team effort with the AUTHOR, Karine Alix, Pat Heslop-Harrison and Graham King being noteworthy contributors. An early draft was produced by Karine Alix which presented the BAC library screening and the FISH work. The AUTHOR enhanced the first draft by detailing the whole BAC sequence analysis and BLASTN comparison of retroelements to GSS. Discussion surrounding the addition of these results was also added by the AUTHOR. The AUTHOR prepared Table 1, 2 and 4 and had a significant input into Table 3, Figs. 1 and 2. Moving from draft format to final document was an iterative process as the document was passed between authors for comment and input. The AUTHOR'S input to the text focused around interpretation of the results and their discussion in the context of *B. oleracea* genomics and implications for comparative genomics. This input is inextricably interwoven with the expertise surrounding TEs delivered by Karine Alix & Pat Heslop-Harrison hence it is not possible in retrospect to highlight a single section of the introduction or discussion written by an individual.

This paper was produced in collaboration with the molecular cytogenetics and genome organisation team at Leicester University and subsequently with

the research unit in plant genetics 'UMR GV Le Moulon', INRA/Univ Paris-Sud/CNRS/AgroParisTech, Gif-sur-Yvette, France, following colleague relocation. This collaboration continued after the publication of this paper and facilitated a subsequent publication, RJ13.

Extensive use of electronic communication methods was made due to the geographical dispersal of parties involved. The AUTHOR was employed as a 'post-doctoral' research scientist (research fellow) at Warwick HRI.

2.5 (RJ13) The CACTA transposon *Bot1* played a major role in Brassica genome divergence and gene expansion. [5]

Hypothesis, scientific approach and conclusions

The AUTHOR hypothesised that a highly replicated C genome specific sequence existed. The basis for this hypothesis was that *in situ* hybridisation results were indicating the presence of a highly replicated repeat that displayed chromosome specificity when hybridised to *B. napus*. Furthermore, long range sequence assemblies in *B. oleracea* were impeded by the presence of uncharacterised, highly repetitive sequences and the absence of available long range sequences was a frustrating hindrance to *B. oleracea* comparative genomic studies. Finally, a widely held assumption is that repetitive elements are a major driver of gene and genome evolution thus an understanding of the TEs within a species, and comparison to those of near relatives, is highly desirable in order to comprehend its evolution. This piece of work aimed to better understand the repetitive units within *B. oleracea*, both at the sequence level and in the wider context of their distribution and prevalence.

At the time this work was carried out Zhang and Wessler, (2004) had carried out a comparative bioinformatics analysis of *A. thaliana* and *B. oleracea* and showed that share largely the same collection of TEs but in differing proportions, with the number of elements of each type being greater in *B. oleracea*. RJ09 had looked at the genomic organisation of selected retrotransposons and an in-depth characterisation of a family of class I TEs in

[5] Karine Alix, Johann Joets, Carol D. Ryder, Jay Moore, Guy Barker, John P. Bailey, Graham J. King & J.S. (Pat) Heslop-Harrison. (2008) Plant Journal. 56(6): pp1030-44

B. oleracea, designated BoS, had been carried out by Zhang and Wessler (2005). The BoS were found to be one of the most diverse Short Interspersed Element (SINE) families from any organism and estimated to be present in ~2000 copies. However, very few of Brassica TEs had been analysed at the molecular level.

This research paper presents the isolation and characterisation of a C genome specific CACTA transposon which we designate *Bot1* (*B. oleracea transposon 1*). It is an in-depth characterisation of a class II TE and we analyse its genomic distribution in the *B. oleracea* (C genome) and the *B. rapa* (A genome). This repetitive sequence is shown to distinguish between the A and C chromosomes in *B. napus* using FISH. We establish the precise section of sequence within *Bot1* which is apparently C-genome specific. Sequence, molecular and cytogenetic analyses show that *Bot1* has proliferated within the C genome and is distinct from TEs in the A and B genomes. This suggests that *Bot1* played a major role in the recent A and C Brassica genome divergence and reinforces the view that the so-called 'junk DNA' is a significant driver of genome and gene evolution and diversification of plant genomes.

Scientific advances enabled

This work is cited as one of two significant recent developments in *B. napus* cytogenetics by Xiong & Pires (2011) because it provides the ability to identify the C genome. NB the other significant development mentioned is chromosome-specific BAC probes delivered in part by RJ07. A BAC clone, BoB014O06, first used in RJ07, had been observed selectively hybridising to specific chromosomes when probed onto *B. napus* chromosomes using

FISH. It is apparently displaying C genome specificity. The *Bot1* TE that we characterise in this piece of work is almost certainly causative of this genome specificity. This BAC clone is now frequently used to facilitate further research in Brassica genomics (e.g. RJ11, RJ12, Mason *et al* 2010, Szadkowski *et al* 2010, Ge *et al* 2009 and Nicolas *et al* 2008 & 2009). This work is also contributing evidence to the wider discussion surrounding genome evolution within plants (Heslop-Harrison, 2012) and more specifically, transposable elements driving the structural, epigenetic and functional modifications during allopolyploidisation and subsequent diploidisation in plants (Parisod *et al* 2009). Finally, our characterisation of *Bot1* adds to a repertoire of established genome characteristics required for corroboration of *B. oleracea* whole genome sequence assembly. Analysis of whole genome sequence comparisons will allow a comprehensive understanding of *B. oleracea* evolution to be attained.

AUTHOR'S contribution and working environment

The AUTHOR conceived the original idea and developed the hypothesis that a highly replicated C genome specific sequence existed. The AUTHOR advocated investigation of TE sequences within three putatively collinear *B. oleracea* BACs as a working strategy. Discussion and collaboration with Karine Alix led to the idea that the C genome specificity being observed using FISH could be attributable to '*Bot1*'. The AUTHOR released the complete sequence of three 'BoB' BACs into the public domain (NCBI GenBank) to accompany the manuscript. The AUTHOR contributed to the estimation of *Bot1*/SLL3 genome copy number from BLASTN results, macro array

hybridisation and scoring, BLAST searches and interpretation of findings in order to ensure sound conclusions were reached.

The first draft of the manuscript was produced by Karine Alix. The AUTHOR instigated the phylogenetic analyses using Brassica sequences presented in Fig. 7 and the addition of copy number estimates obtained by BLASTN presented in Table 3. Both of these analyses were carried out by Jay Moore. Compilation of the manuscript was a team effort with the AUTHOR, Karine Alix, Pat Heslop-Harrison and Graham King being noteworthy contributors. Moving from draft format to final document was an iterative process as the document was passed between authors for comment and input. Beyond instigating the addition of the two sequence analyses the AUTHOR'S input to the manuscript focused around improving the overall quality and readability of the text and the figures and their discussion in the context of *B. oleracea* genomics and implications for comparative genomics.

This publication is the result of continued collaboration of the team which produced RJ09. Extensive use of electronic communication methods was made due to the geographical dispersal of parties involved. The AUTHOR was employed as a 'post-doctoral' research scientist (research fellow) at Warwick HRI.

3 Conclusions

Taken as a coherent, sequential body of work these publications demonstrate consistent advancements in the field of comparative *B. oleracea* genomics by the identification of unique genome characteristics in the pursuit of a comprehensive understanding of genome evolution.

The AUTHOR has analysed the *B. oleracea* genome at both the macro and micro scale and the results are widely used by the scientific community. Taken independently each paper presents a significant advancement of understanding and challenges the boundaries of scientific knowledge and debate. RJ05 highlighted that the macro collinearity between the *B. oleracea* genome and the *A. thaliana* genome is often considerable but segmental and inconsistent. This has been borne out by subsequent whole genome sequencing of the *B. rapa* genome (2011). RJ10 increased the resolution of the large segmentally duplicated and inverted collinear region of *B. oleracea* C6 revealed by RJ05. This genomic characterisation has subsequently facilitated research studies in *B. napus*, highlighting the conserved nature of the polyploid genome (Howell *et al* 2008, Wang *et al* 2009). The assignment and orientation of *B. oleracea* chromosomes to linkage groups achieved in RJ07 is the definitive work on this topic and as such underpins all subsequent research involving the C genome. The investigation of copy number, genomic organisation, isolation and characterisation of transposons presented in RJ09 and RJ13 not only enhances our knowledge of the C genome but in doing so also allows deductions to be made regarding Brassica genome evolution using comparative genomics. Furthermore, the demonstration that the repetitive *Bot1* sequence is C genome specific is

recognised as a key development in *B. napus* molecular cytogenetics (Xiong & Pires, 2011). This work is informing the contemporary debate surrounding transposable elements driving the structural, epigenetic and functional modifications during allopolyploidisation (Heslop-Harrison, 2012, Parisod *et al* 2009). Both individually and as a body of work these publications substantially advance the fields of comparative, Brassica and genomic research.

Towards a comprehensive understanding of Brassica genome evolution

A comprehensive understanding of *B. oleracea* evolution will eventually be attained through analysis of whole genome sequence comparisons. However, an assembled whole genome sequence for *B. oleracea* has yet to be published. The advent of "next-generation" sequencing technology, which uses a shotgun approach, has made it possible to cost effectively generate whole genome sequence data in a matter of days. Many data sets for different *B. oleracea* genotypes exist (e.g. http://flora.acpfg.com.au/tagdb/cgi-bin/index). However, accurate *de novo* assembly of the short (~150bp) sequence reads into large contigs, and eventually chromosomes, is beyond current computational capabilities unless some framework is available to reduce the complexity. In order to achieve a verified whole genome sequence, predicted assemblies must be subjected to an iterative verification process to ensure they depict reality. This corroboration process is only possible if a repertoire of established genome characteristics is available. The inverted segmental duplication characterised in RJ05 and RJ07, the repetitive elements characterised in RJ09 and RJ013, the BAC library and

whole BAC sequences and the ability to orientate chromosomes in combination with chromosome to linkage group alignments achieved by RJ10 are all works of reference for the *B. oleracea* genome and hence essential during the sequence verification process. *De-novo* assemblies are orders of magnitude slower, more complex and memory intensive than 'mapping assemblies'. A mapping assembly aligns individual reads against an existing backbone sequence, hence building a sequence that is similar but not necessarily identical to the backbone sequence. The data assembly process for *B. oleracea* may be augmented using the draft whole genome sequence of *B. rapa* (The *Brassica rapa* Genome Sequencing Project Consortium, Aug. 2011) as a backbone in a mapping assembly.

The multinational *B. rapa* Genome Sequencing Project (BrGSP) began using end-sequenced and chromosome-specific BAC by BAC sequencing in 2003. Mun *et al* (2010) published the near-complete sequence of *B. rapa* chromosome A3 compiled using 348 overlapping and physically mapped BACs (Mun *et al* 2008). The physical mapping of the BACs provided the critical established genome characteristic links required for verification in this instance. "Next-generation" sequencing technology was used develop the draft whole genome sequence of *B. rapa* (The *Brassica rapa* Genome Sequencing Project Consortium, 2011). Their *de novo* assembly was verified and aided by checking against previously established genome characteristics in *B. rapa* and *B. napus* A genome. Where genome information was not available in Brassica, contig order and/or orientation was inferred based on evidence of conserved collinearity with the *A. thaliana* gene order. This suggests that *B. oleracea* whole genome sequence assembly will not only be

facilitated by the draft *B. rapa* sequence but also by its more distant relative, *A. thaliana*.

The availability of a complete Brassica genome sequence is not only likely to accelerate the compilation of additional Brassica genomes but, in doing so, bring new comparative genomic possibilities. For example, whole genome alignments between the *B. rapa* (AA), B. nigra (BB) and *B. oleracea* (CC) genomes, each of these to *A. thaliana* and to the allopolyploid genomes of *B. napus* (AACC), *B. carinata* (BBCC) and *B. juncea* (AABB). Annotation and analysis of these comparative alignments would allow us to challenge our current understanding of their genome evolution whilst underpinning the genetic improvement of Brassica oil and vegetable crops. If a comprehensive understanding of the evolution of these cruciferous species were to be attained it would undoubtedly allow hypotheses to be tested regarding the genome evolution of other plant species.

Attaining robust conclusions from these comparisons is dependent upon the accuracy of the whole genome sequence assemblies. *De novo* assembly in combination with mapping assembly still requires verification of predicted alignments with chromosomes and/or robust genetic linkage maps to ensure unequivocal assignment and orientation of sequence scaffolds. In the case of *B. oleracea*, and consequently the C genome, the AUTHOR'S published work will underpin the assembly and verification processes.

Of broader significance, the fundamental methodology of the verification process developed through this work has the potential to enable accurate whole sequence assembly in other complex genomes.

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5 Appendix

5.1 Percentage Contribution of AUTHOR to Submitted Papers

TABLE 2: Percentage contribution of AUTHOR for each presented publication.

Paper	Ref. code*	Journal	Impact factor	% contribution		
				Original idea	Execution of work	Writing
Ryder *et al*	RJ05	Genome	1.662	30%	90%	70%
Howell *et al*	RJ07	Genetics	4.087	25%	50%	30%
Howell *et al*	RJ10	Genome	1.662	20%	40%	30%
Alix *et al*	RJ09	Plant Molecular Biology	4.149	25%	25%	35%
Alix *et al*	RJ13	The Plant Journal	6.948	75%	[a]10%	30%

[a] AUTHOR facilitated and directed of work of colleagues by sharing results to ensure coherent conclusions. This is not reflected in the % estimates shown.
* See Appendix 5.4 - Complete list of the AUTHOR'S published works and Appendix 5.5 - Citation data for AUTHOR'S published Works

5.2 Corroborating statements from co-authors.

Letters confirming the input of the AUTHOR from all key co-authors are included below. These universally confirm the statements made in this document and confirm the AUTHOR'S significant scientific contributions to these papers.

Professor Graham King
Director
Southern Cross Plant Science
Southern Cross University
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LISMORE NSW 2480
Ph: +61(0) 2-6620 3010
E-mail: graham.king@scu.edu.au

July 4th 2012

To whom it may concern:

Re: Carol Ryder, thesis **"Genomics of *Brassica oleracea*", submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy by Publication.**

I have recently had the opportunity to read the Covering Document prepared by Carol Ryder (June 2012) for her thesis submission. I am able to confirm that the presentation of the circumstances, research environment and allocation of effort (particularly that summarised in Appendix 5.1.) are consistent with my recollection and understanding.

I am very happy to corroborate the statements and interpretation made by Carol Ryder, including her assessment of the impact of the research.

Regards

Graham King BSc, PhD

Director,
Southern Cross Plant Science,
Southern Cross University,
Lismore
NSW 2470
Australia

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Patron: Her Majesty The Queen

February 27th 2011

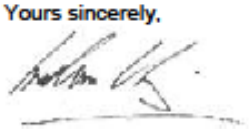
To whom it may concern:

re: Carol Ryder, thesis "Genomics of *Brassica oleracea*", submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy by Publication.

I have had the opportunity to read the thesis prepared by Carol Ryder. I am very happy to confirm that Section 3 is in full accordance of my understanding of the extent of her contribution to the research and the circumstances under which the work was carried out.

Carol has made significant contributions to the development of *Brassica* genomics over the years, which she has described well in her thesis. She built on her initial involvement in optimising and refining molecular assays for distinguishing self-incompatibility alleles, by successfully generating the first *Brassica oleracea* BAC library which formed the basis for a wide range of projects in the UK and elsewhere. Amongst these, Carol has carried out detailed comparative genomics work, and acquired an in depth knowledge of the properties of *Brassica* genome organisation. Her thorough knowledge of the relevant experimental resources and attention to detail has been invaluable to myself and colleagues throughout the world, and ensured that a number of key areas of research were able to progress – in particular the integration of cytological and genetic maps which now forms the basis for understanding how the *Brassica* C genome relates to the chromosomes, which is essential for ongoing sequencing efforts. I was very happy to see Carol develop into an independent researcher, and in particular have been impressed at how she has ensured that productive collaborations have come to fruition in a number of areas.

Yours sincerely,



Graham King, BSc, PhD

Deputy Scientific Director of Centre for Crop Genetic Improvement
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from March 2011:
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UNIVERSITY OF BIRMINGHAM

School of Biosciences

Susan Armstrong
Senior Lecturer in Genetics

Direct Tel: 0121 414 6485
Direct Fax: 0121 414 5925
Email: s.j.armstrong@bham.ac.uk
25th June 2012

Statement regarding the contribution of Carol Ryder to our joint papers;

E.C. Howell, G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, E.P. Kop, C.D. Ryder, G.R. Teakle, J.G. Vicente, S.J. Armstrong. (2002) Integration of a cytogenetic map with a genetic linkage map of *Brassica oleracea.* Genetics. 161(3): pp1225-1234

E.C. Howell, G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, C.D. Ryder, S.J. Armstrong. (2005) Physical organization of the major duplication on *Brassica oleracea* Chromosome O6 revealed through fluorescence *in situ* hybridization with *Arabidopsis* and *Brassica* BAC probes. Genome. 48(6): pp1093-1103.

I understand that Carol has been asked to provide the % breakdown that she has provided for her contribution to our joint papers, I fully agree with her statement and I would like to reiterate my previous comments as follows;

I am delighted to provide a statement of Carol's contribution to our joint papers. The statement that she provides recording her contribution to each of our two joint papers is entirely correct and the quality of the work she produces is always of a high standard. In the first paper, Carol was instrumental in producing the genetically mapped probes which were essential for us to link the cytogenetical and physical maps for *Brassica oleracea*. In the second paper we relied on Carol's knowledge of the collinearity between *Brassica* and *Arabidopsis*.

Susan Armstrong

Susan Armstrong

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UNIVERSITY OF BIRMINGHAM
School of Biosciences

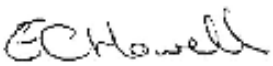
Elaine Howell Ph.D.
Research fellow
Tel. 0121 414 5909

Re: Comparative Genomics of *Brassica oleracea* by **Carol Ryder**, University of Warwick.

I have been a Post-doctoral Research Fellow at the University of Birmingham for over 20 years, specialising in cytogenetics of *Brassica oleracea* and *B. napus*. I am the first author of the papers referred to in Section 2.2, (RJ07) Integration of a cytogenetic map with a genetic linkage map of *B. oleracea* , and Section 2.3, (RJ10) Physical organization of the major duplication on *B. oleracea* Chromosome O6 revealed through fluorescence *in situ* hybridization with *Arabidopsis* and *Brassica* BAC probes.

I confirm that the statements regarding Carol Ryder's involvement in the research resulting in these publications are correct. The research relied on the high quality BoB BAC library which Carol had already produced. Carol's experience of working with BAC libraries, her extensive knowledge of the genetic maps of both *Arabidopsis* and *B. oleracea*, and her awareness of the difficulties of working with the triplicated nature of the *B. oleracea* genome were critical to the success of this research. I collaborated closely with Carol throughout the three year funding period and beyond. As well as having considerable practical involvement, Carol was able to analyse the implications of the results and inform our discussions on the way forward, contributing consistently at a post-doctoral level. The success of the project depended on frequent discussions between the two of us, and a mutual respect and trust in each other's expertise underpinned this dialogue.

Carol has made a significant contribution to the field of Brassica genomics and I found the experience of working with her both rewarding and a pleasure.



Elaine Howell

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9th July 2012

To whom it may concern.

Re Carol Diana Ryder; PhD submission by publication

Dear Sir or Madam,

This is to confirm that Carol Diana Ryder's contribution to the following two papers was precisely as she states in her submission in Section 2.2 pages 15-19.

1. (RJ07) Howell, EC, Barker, GC, Jones, GH, Kearsey, MJ, King, GJ, Kop, EP, Ryder, CD,
Teakle, GR, Vicente, JG, Armstrong, SJ. (2002) Genetics 161(3): 1225-1234

and Section 2.3 pages 20-23

2. (RJ10) E.C. Howell, G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, C.D. Ryder, S.J.
Armstrong. (2005) Genome. 48(6): pp1093-1103.

Her % contributions to towards the conception, execution and writing of the work are accurately represented in Appendix 5.1 on page 47.

Her contribution was extremely valuable and required considerable skills in Brassica physical mapping, BAC handling and consultation with other scientists. The project would not have been possible without her contributions. The work was seminal in Brassica genetics leading not only to the first clear physical map of Brassica oleracea but also linked it to the sequenced model, Arabidopsis.

Yours sincerely,

Professor Mike Kearsey

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THE UNIVERSITY OF
WARWICK

Carol Ryder
353 Closson Rd
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NY 12302-6747
USA

28th June 2012

Dear Carol

I am responding to your request for corroboration of your contribution to the joint publications that you have submitted for consideration for a 'PhD by publication.' The two papers for which I am a co-author are:

1) C.D. Ryder, L.B. Smith, G.R. Teakle & G.J. King. (2001) Contrasting genome organisation: Two regions of the Brassica oleracea genome compared with collinear regions of the Arabidopsis thaliana genome. Genome. 44: pp808-817

2) E.C. Howell, G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, E.P. Kop, C.D. Ryder, G.R. Teakle, J.G. Vicente, S.J. Armstrong. (2002) Integration of a cytogenetic map with a genetic linkage map of Brassica oleracea. Genetics. 161(3): pp1225-1234

My contribution to Ryder et al (2001) was the generation of the genetic map. I worked closely with Carol in this work and can verify that Carol performed/co-ordinated the data generation and analysis and had a significant role in writing the paper.

In the second paper by Howell et al (2002), HRI worked in collaboration with Sue Armstrong and Elaine Howell at Birmingham University who provided the expertise in fluorescent in situ hybridisation (FISH). My role was again to provide map information for a number of the BAC clones used to link the cytogenetic map to the genetic map. In this work Carol was the primary co-ordinator between the Birmingham and HRI research groups. Carol made a significant contribution to mapping a number of the BAC clones used and was the person with the primary role of interpreting the data which made this publication possible. Again Carol had a significant contribution to writing the paper.

I confirm that Table 2 in Appendix 5.1 represents a fair attribution of Carol's input into this work.

I have worked with Carol for a number of years and have always been impressed with her level of knowledge, technical expertise, intellectual input and the reliability of her data. Carol has had a very significant input into a number of projects and has provided a key collaborative role with external researchers on a number of occasions. I consider that Carol's work and capabilities are readily commensurate with a higher level degree and fully support her submission for a 'PhD by publication.'

With best regards

Gd Teakle.

Dr. Graham Teakle

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Carol Dunsdon

From: Heslop-Harrison, Pat (Prof.) <phh4@leicester.ac.uk>
Sent: Thursday, June 28, 2012 3:18 PM
To: Carol Dunsdon
Subject: RE: Carol Ryder - PhD by publication

Hello Carol,

I received this in hospital with hepatitis E. Now home but in no state to do much so I hope this is sufficient.

I fully agree with the statements in your letter about contributions to the papers. without your considerable and significant contributions we would not have been able to do this work.

Best wishes

Pat
Pat Heslop-Harrison.

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Carol Ryder

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18 February, 2011

Dear Carol,

I am delighted to hear that you are applying for a PhD by publication.

As you know, I am coauthor (and last author, a position conventionally indicating some role in coordination of the work), of two of your joint authorship papers (4 and 5; Alix et al., 2005 and 2008). In both of these papers, you played a very significant , distinctive and scientifically-based part in the research reported from the conception of how the resources you have developed could be used (including that our projects would find the transposon-containing BACs you had identified of particular interest and relevance), to the novel technical work based on your genomics expertise and knowledge of the *Brassica oleracea* genome (both informatics and hybridization), through to the genomics based concepts in writing up and publication of information about the sequences. With the combination of this type of genomics work requiring diverse expertise, and the publications in high profile Journals (Plant Journal; Plant Molecular Biology) requiring considerable data and significant conclusions, it is normal to have multiple authors.

I am very happy to confirm that your substantial and intellectual role in the papers is accurately stated in your submission **3.4 (RJ09)** and **3.5 (RJ13).** Without your excellent input and work, the project would not have proceeded in the direction that was possible, leading to two valuable papers with novel conclusions about Brassica genome evolution.

Yours sincerely,

J.S. Heslop-Harrison

Professor of Molecular Cytogenetics and Cell Biology.

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Gif-sur-Yvette, 27.06.2012

To Whom It May Concern

I am writing this letter in support of Carol Ryder's submission for a 'PhD by publication'.

I met Carol Ryder when I was a postdoctoral fellow in Prof. Pat Heslop-Harrison's laboratory at the University of Leicester, in 2001. My project aimed to characterize *Brassica* retrotransposons at both the cytogenetic and genomic levels, to evaluate the role of such repetitive elements in shaping the *Brassica* genome along with polyploidisation and speciation events. We thus set up a fruitful scientific collaboration with Carol Ryder and Graham King at WHRI, Wellesbourne, to achieve this objective.

The first set of results we obtained dealt with the genomic organization of retrotransposons in the *B. oleracea* genome. Carol Ryder was in charge of the *B. oleracea* library 'BoB' she developed and characterized, and helped us in exploiting this genomic resource (hybridization and scoring), while she took charge of analyzing the scoring data and molecular experiments for characterizing specific TEs. We published these results in Plant Molecular Biology (IF 4.15) – Alix et al. 2005, RJ09.

Our collaboration was then pursued when I moved to the research unit 'UMR GV Le Moulon', INRA/Univ Paris-Sud/CNRS/AgroParisTEch, Gif-sur-Yvette, France, where I got a permanent position as a Lecturer at AgroParisTech. We finalized our study on the C-genome-specific transposon *Bot1*, thanks to Carol Ryder's enthusiasm and personal involvement. She conceived the idea to analyze 3 BAC sequences from a triplicated *B. oleracea* region, which allowed the full genomic description of the CACTA transposon *Bot1*. This lead to a second paper which was published in The Plant Journal (IF 6.95) – Alix et al. 2008, RJ13.

Therefore, I fully agree with Carol Ryder's statements reported in her document, notably with the description of the extent of her contribution to the research project we conducted (p.24-32). In conclusion, I recommend validation of her 'PhD by publication' without reservation.

Sincerely,

Dr Karine ALIX
Lecturer, AgroParisTech
UMR Génétique Végétale Le Moulon
INRA/Univ Paris-Sud/CNRS/AgroParisTech
F-91190 Gif-sur-Yvette, France

Dr. Jay Moore
Senior Research Fellow in Bioinformatics
Systems Biology Centre
Coventry House
University of Warwick
Coventry
CV4 7AL

3rd July 2012

Dear Carol,

I am very happy to confirm that I agree with your summaries of the papers we worked on together, of your own role in the research and on the proportions of the papers directly attributable to you. In my opinion your contribution to the field of Brassica research has been original and substantial.

Best wishes

Jay Moore

Dr Lee Smith
Reader in Genetic Endocrinology and Principal Investigator
MRC Centre for Reproductive Health
University of Edinburgh
The Queen's Medical Research Institute
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Edinburgh
EH16 4TJ
UK

Lee.Smith@ed.ac.uk

23rd June 2012

Carol Ryder
353 Closson Rd
Glenville, NY
12302-6747
USA

Dear Carol

Regarding our joint publication, namely:

C.D. Ryder, L.B. Smith, G.R. Teakle & G.J. King. (2001) Contrasting genome organisation: Two regions of the Brassica oleracea genome compared with collinear regions of the Arabidopsis thaliana genome. Genome. 44: pp808-817

This work was carried out whilst I was a PhD student at Warwick University. The focus of my thesis was the characterisation and mapping of BoCAL and BoAP1 genes and their effect on cauliflower curd morphology. Copies of these genes locate within the regions investigated in the above publication. I confirm that I had very minimal input into the execution, interpretation and documentation of this *B. oleracea/A. thaliana* collinearity study. Although it is some time ago now it is my recollection that you executed the vast majority of the work involved in this publication.

Best regards,

Lee Smith

MRC Centre For Reproductive Health; University of Edinburgh
The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ UK
telephone +44 (0) 131 242 9100 fax +44 (0) 131 242 6231

Bayer CropScience

To:
Carol Ryder
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USA

27th June 2012

Dear Carol,

Re: Submission for doctorate by publication.

Thank-you for your recent e-mail. I have examined the covering document you sent me and I am happy to confirm that I agree with the statements in section 2.2 and appendix 5.2 regarding the extent of your individual contribution to the publication on which I am a co-author, namely:

Integration of a cytogenetic map with a genetic linkage map of *Brassica oleracea*. Genetics. (2002) 161(3): pp1225-1234

I wish you all the very best with your PhD submission and examination. The award would be very much deserved after all your years of research.

Yours sincerely,

Frik Kop (PhD)

Dr Joana Vicente
School of Life Sciences
The University of Warwick
Wellesbourne Campus
Warwick CV35 9 EF

Carol Diana Ryder
353 Closson Rd, Glenville
NY 12302-6747

27th June 2012

Dear Carol,

I am pleased to hear that you are completing your application for a PhD by publication. Your contribution to the field of Brassica genomics is very important and should be acknowledged.

You have worked at the Wellesbourne Campus, now part of the School of Life Sciences (previously Warwick HRI and Horticulture Research International) in different projects, but always following the theme of *Brassica* genomics for over 10 years. Your work also extends to rosaceous species. You have published a range of papers in high profile journals that have now received nearly 1000 citations. In addition, the resources that you have created (including a *Brassica oleracea* BAC library constructed in Texas A&M University) have been used by a large number of researchers including myself.

I am a co-author of the paper:

Howell, EC, Barker, GC, Jones, GH, Kearsey, MJ, King, GJ, Kop, EP, Ryder, CD, Teakle, GR, Vicente, JG, Armstrong, SJ. (2002) Integration of the cytogenetic and genetic linkage maps of *Brassica oleracea*. Genetics 161(3): 1225-1234

I can confirm Carol's contribution for this work done in collaboration with E. Howell and S. Armstrong from the Birmingham University. Carol made a contribution to this collaborative work at all stages, from the idea to writing the paper. Carol chose candidate FISH probes that were used to identify each chromosome and communicated frequently with E. Howell. Carol's contribution was essential for the success of this work.

With compliments,

Joana Vicente

5.3 Career, research and working environment

The AUTHOR'S career in plant molecular biology commenced in 1993 as an entry level research scientist. A series of roles with increasing responsibility led to a promotion to a higher employment band in 2002. The job evaluation process which led to this promotion judged the role to be performing "innovative research and development work involving aims which are broadly defined or only defined as an end product. Where a team is involved it will not necessarily be organised hierarchically." Consequently, for ten years preceding the submission of this covering document the AUTHOR was employed as a 'post-doctoral' research scientist (research fellow) on a series of successful research projects. Extended periods have been spent working on secondment in other laboratories across Europe and in the USA. The AUTHOR has 15 plant genetics and genomics publications in peer reviewed journals. The AUTHOR has addressed a broad range of plant genetics and genomics questions. A particular interest in both *Brassica* and the rosaceous species has been retained throughout her research career, one often taking precedence over the other but neither ever disappearing completely. Whilst work on these two crop types has never amalgamated into the same research project, knowledge and techniques have routinely been taken from one species and applied to the other. A common theme throughout has been the use of comparative genomics to study plant evolution. From this broad body of work the AUTHOR selected five highlights that show this common theme for *B. oleracea*. However, the publications not selected for presentation serve to provide additional evidence of the AUTHOR'S research background and contribution to plant genomics (Appendix 5.4, 5.5 & 5.6).

The research has generally been carried out in a team environment with individuals working together to orchestrate techniques and approaches to focus upon a research topic, test ideas and discuss principles. Working relationships with co-authors vary from those within the same research team (e.g. line manager/PI, contemporaries and PhD students) through to close collaboration with external research groups. Relationships also vary for an individual depending upon which publication is being discussed, for example, a co-author common to all publications presented, changed from being line manager/PI to external collaborator in 2005.

5.4 Complete list of the AUTHOR'S published works

(KEY: RJ## are peer reviewed journal papers, NJ## are non-peer reviewed journal papers, LA## are lecture abstracts, CA## are conference poster abstracts, R## are reports, S## are sequence releases.)

RJ01 J.Brace, C.D. Ryder & D.J. Ockendon (1994) Identification of S-alleles in *Brassica oleracea. Euphytica 80:229-234.*

RJ02 Roche, P.A., Alston, F.H., Maliepaard, C.A., Evans, K.M., Vrielink, R., Dunemann, F., Markussen, T., Tartarini, S., Brown, L.M., Ryder, C., King, G.J. (1997) RFLP and RAPD markers linked to the rosy leaf curling aphid resistance gene *(Sd_1)* in apple. Theoretical and Applied Genetics 94:528-533

RJ03 Maliepaard, C, Alston, FH, van Arkel, G, Brown, LM, Chevreau, E, Dunemann, F, Evans, KM, Gardiner, S, Guilford, P, van Heusden, AW, Janse, J, Laurens, F, Lynn, JR, Manganaris AG, den Nijs, APM, Periam, N, Rikkerink, E, Roche, P, Ryder, C, Sansavini, S, Schmidt, H, Tartarini, S, Verhaegh, JJ, Vrielink-van Ginkel, M, King, GJ. (1998) Aligning male and female linkage maps of apple (Malus pumila Mill.) using multi-allelic markers. Theoretical & Applied Genetics 97:60-73.

RJ04 Sosinski, B, Gannavarapu' M, Hager L.D., Beck' L.E., King G.J., Ryder, C.D., Rajapakse, S., Baird, W.V., Ballard, R.E., Abbott, A.G. (2000) Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theoretical & Applied Genetics 101: (3) 421-428

RJ05 Ryder CD, Smith, LB, Teakle, GR & King GJ (2001) Contrasting genome organisation: Two regions of the *Brassica oleracea* genome compared with collinear regions of the *Arabidopsis thaliana* genome. GENOME 44, 808-817.

RJ06 Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchini, R., van de Weg, E., & Gessler, C., (2002) Development and characterisation of 140 new microsatellites in apple (Malus x domestica Borkh.). Molecular Breeding 10(4): 217-241

RJ07 Howell, EC, Barker, GC, Jones, GH, Kearsey, MJ, King, GJ, Kop, EP, Ryder, CD, Teakle, GR, Vicente, JG, Armstrong, SJ. (2002) Integration of the cytogenetic and genetic linkage maps of *Brassica oleracea.* Genetics 161(3): 1225-1234

RJ08 M. J. Aranzana, A. Pineda, P. Cosson, E. Dirlewanger, J. Ascasibar, G. Cipriani, C. D. Ryder, R. Testolin, A. Abbott, G. J. King, A. F. Iezzoni, and P.

Arús (2003) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theoretical & Applied Genetics 106: 819-825

RJ09 Alix, K, Ryder, CD, King, GJ, Moore J & Heslop-Harrison JS. (2005) The genomic organization of retrotransposons in *Brassica oleracea*. Plant Molecular Biology 59(6): 839-851

RJ10 Howell, EC, Barker, GC, Jones, GH, Kearsey, MJ, King, GJ, Ryder, CD, Armstrong, SJ. (2005) Physical organization of the major duplication on *Brassica oleracea* Chromosome O6 revealed through fluorescence *in situ* hybridization with *Arabidopsis* and *Brassica* BAC probes. Genome 48(6): 1093-1103

RJ11 M. Leflon, F. Eber, J.C. Letanneur, L. Chelysheva, O. Coriton, V. Huteau, C.D. Ryder, G. Barker, E. Jenczewski and A.M. Chèvre. (2006) Pairing and recombination at meiosis of *Brassica rapa* (AA) x *Brassica napus* (AACC) hybrids. Theoretical & Applied Genetics 113:1467-1480.

RJ12 Stéphane D Nicolas, Guillaume Le Mignon, Frédérique Eber, Olivier Coriton, Hervé Monod, Vanessa Clouet, Virginie Huteau, Antoine Lostanlen, Régine Delourme, Boulos Chaloub, Carol D Ryder, Anne Marie Chèvre and Eric Jenczewski. (2007) Homoelogous recombination plays a major role in chromosome rearrangements that occur during meiosos of *Brassica napus* haploids. Genetics 175: 487–503.

RJ13 Karine Alix, Johann Joets, Carol D Ryder, Jay Moore, Guy Barker, John P Bailey, Graham J King & J S (Pat) Heslop-Harrison. (2008) The CACTA transposon Bot1 played a major role in Brassica genome divergence and gene proliferation. Plant Journal. 56(6): 1030-44

RJ14 Volkan Cevik, Carol D. Ryder, Alexandra Popovich, Kenneth Manning, Graham J. King and Graham B. Seymour. (2010) A FRUITFULL-like gene is associated with genetic variation for fruit flesh firmness in apple (*Malus domestica* Borkh.) Tree Genetics & Genomes 6(2): 271-279

RJ15 Graham B. Seymour, Carol D. Ryder, Volkan Cevik, John P. Hammond, Alexandra Popovich, Graham J. King, Julia Vrebalov, James J. Giovannoni and Kenneth Manning. (2010) A SEPALLATA gene is involved in the development and ripening of strawberry (*FragariaXananassa* Duch.) fruit, a non-climacteric tissue. J. Exp. Bot. (2011) 62(3): 1179-1188

NJ01 Maliepaard, C., Alston, F. H., van Arkel, G., Brown, L. M., Chevreau, E., Dunemann, F., Evans, K. M., Gardiner., S., Guilford, P., van Heudsen, S., Janse, J., Laurens, F., Lynn, J. R., Manganaris, S., den Nijs, A. P. M., Periam,

N. W., Rikkerink, E., Roche, P., Ryder, C. D., Sansavini, S., Schmidt, H. L., Tartarini, S., Verhaegh, J., Vrielink, R., King, G.J., (1999) The European Apple Map. Acta Horticulturae 484:325-330

NJ02 Armstrong SJ, Howell EC, Fransz P, Jones GH, Kearsey MJ, King GJ, Kop E, Ryder CD, Teakle GR & Vicente JG. (2001) Integrating the Genetic and Physical Chromosome Maps of *Brassica oleracea* var *alboglabra.* Acta Horticulturae 539: 77-82

LA01 Armstrong, SJ, Howell, EC, Jones GH, Kearsey MJ, Ryder CD, King GJ (2001) Integrating the genetic and physical chromosome maps of *Brassica oleracea*. Brassica workshop, Plant & Animal Genome IX Conference, San Diego

LA02 King, GJ, Barker, GC, Naylor R, Patel, D, Ryder, CD, Bancroft I, Beynon J, Kearsey MJ, Scott R, Trick M. Development and comparative analysis of a *B. oleracea* physical map, anchored to the arabidopsis genome. Large-Insert DNA Libraries and Their Applications: Workshop. Plant & Animal Genome Conference X, San Diego. Jan 2002

LA03 Ryder CD, Barker GC, Howell EC, King GJ. Long-range sequence comparison of a *Brassica oleracea* gene family to Arabidopsis. Procedings of the second Plant GEMs (and the fourth GARNet Functional Genomics) Meeting, 2-6 September 2003, York, UK.

LA04 Graham J King, Graham R Teakle, Charlotte J Allender, Guy C Barker, David A Pink, Carol D Ryder. From trait to genome: characterising brassica diversity. Brassicas workshop. Plant & animal Genome XII, San Diego Jan 10-14th 2004.

LA05 Carol Ryder. The non-human genome project. From model plant to crops; Brassica genomics. BA festival of science, Exeter University. 4-11 Sept 2004

LA06 Carol Ryder, Guy Barker, Graham Teakle, James Lynn, David Pink, Jean-Charles Deswarte, Graham Farquhar, Philip White and Andrew Thompson. Water-Use-Efficiency Genes in *Brassica oleracea*. Brassica2008, 5th ISHS International Symposium on Brassicas and 16th Crucifer Genetics Workshop, 8 - 12 September 2008, Lillehammer, Norway

LA07 Andrew Thompson, Carol Ryder, Howard Hilton, James Lynn, Graham Teakle, Dave Pink. Genetic control of water use efficiency in *Brassica oleracea.* UK-BRC meeting, JIC, Norwich, 7th May 2009.

CA01 Periam, N.W., Ryder, C., Brown, L.M., & King, G.J. (1996) Application of microsatellite markers to linkage mapping in *Malus*, based on a diverse set of crosses. Eucarpia Symposium on Fruit Breeding & Genetics, Oxford, 1st-6th Sept. 1996.

CA02 Ryder, C., Edwards, K., Periam, N. & King, G.J. (1996) Development of microsatellite markers in the *Rosaceae*. Eucarpia Symposium on Fruit Breeding & Genetics, Oxford, 1st-6th Sept. 1996.

CA03 King, G.J., Brown, L.M., Lynn, J.L., Periam, N., Ryder, C.D. (1997) Marker development, map exploitation, experimental design and database management arising from the European apple genome mapping project (1993-1996). Plant & Animal Genome V, San Diego, Jan 12-16 1997.

CA04 Ryder, C.D, King, G.J., Armstrong, S. (1998) Use of a BAC library to facilitate comparative genetic and physical analysis of the *Brassica oleracea* genome. 11th Crucifer Genetics Workshop, Ste Gabriel, Sept 1998.

CA05 Smith, L.B., Teakle, G.R., Armstrong, S.J., Ryder, C.D., McClenaghan, E.R., Sebastian, R., Yanofsky, M.F., King, G.J. (1998) Genomic organisation of homeotic genes and their role in *Brassica oleracea* morphology. 11th Crucifer Genetics Workshop, Ste Gabriel, Sept 1998.

CA06 Teakle, G. R., Smith, L. B., Mcclenaghan, E. R., Ryder, C. D., (Sebastian, R.) & King, G. J. (1998). Molecular genetic regulation of *Brassica oleracea* morphological variation. Paper presented at The role of developmental control genes in the evolution of plant adaptation, ESF Workshop, Stockholm, Sweden, 21-24 August 1998,

CA07 Wilkes, T., Leckie, D., Cogan, N. O. I., Ryder, C., Breeds, S. E., Gordon, P.L., (Parkin, I.), Bittner-Eddy, P., (Williams, K.), Beynon, J. L., Crute, I. R. & Holub, E. B. (1998). Application of knowledge about resistance gene organisation in *Arabidopsis thaliana* for genetic improvement of *Brassica oleracea*. Proceedings, 7th International Congress of Plant Pathology, Edinburgh, 9-16 August 1998,

CA08 Graham J. King, M.J. Bennett, S. May, G. McEwan, C.D. Ryder, A. Sarjeant, L.B. Smith, G.R. Teakle (1999) Comparing genetic & physical organisation of gene families affecting plant development within *Brassica* & *Arabidopsis.* Lecture and abstract 10th International Rapeseed Congress, Canberra, Australia, Sept, 1999

CA09 King, G.J., McEwan, G., Ryder, C.D., Smith, L.B., Teakle, G.R., Vicente, J. (1999) Determining the organisation of gene families in the *Brassica oleracea* genome through accurate analysis of allelic differences and a large insert BAC library. Plant & Animal Genome VII conference, San Diego, Jan 1999

CA10 Armstrong, S. J., Howell, E. C., Fransz, P., Jones, G. H., Kearsey, M. J., King, G. J., Kop, E., Ryder, C. D., Teakle, G. R. & Vicente, J. G. (2000) Integrating the genetic and physical chromosome maps of *Brassica oleracea* var. alboglabra. 3rd International ISHS Symposium on Brassicas and 12th Crucifer Genetics Workshop, Sept 2000

CA11 Howell, EC, Jones GH, Kearsey MJ, King GJ, Ryder CD, Vicente, JG, Armstrong SJ (2000) Integrating the genetic and physical maps of *Brassica oleracea.* 3rd International ISHS Symposium on Brassicas and 12th Crucifer Genetics Workshop, Sept 2000

CA12 Ryder CD, Smith LB, Naylor R, Barker G, King GJ (2000) Genome organisation: two regions of the *Brassica oleracea* genome compared with colinear regions of the *Arabidopsis thaliana* genome. 3rd International ISHS Symposium on Brassicas and 12th Crucifer Genetics Workshop, Sept 2000

CA13 Teakle, GR, Kop E, Smith LB, Ryder CD, McClenaghan, ER & King, GJ (2000) Molecular Genetic Regulation of Early Floral Development in *Brassica oleracea.* 3rd International ISHS Symposium on Brassicas and 12th Crucifer Genetics Workshop, Sept 2000

CA14 Barker G, Patel D., Naylor, R., Ryder, CD, Beynon, J., and King GJ.(2000) Towards a contiguous physical map of the Brassica C genome: A valuable resource for the Brassica community. 3rd International ISHS Symposium on Brassicas and 12th Crucifer Genetics Workshop, Sept 2000

CA15 Allen, T, Ryder, CD, King, GJ, Bennett, MJ (2000) Organisation & allelic analysis of the LAX gene family in *Brassica oleracea.* 3rd International ISHS Symposium on Brassicas and 12th Crucifer Genetics Workshop, Sept 2000

CA16 Teakle, GR, Kop, EP, Smith, LB, Ryder CD, McClenaghan ER, King, GJ (2000) Molecular Genetic Regulation of Early Floral Development in *Brassica oleracea*. 18th International Congress of Biochemistry and Molecular Biology - Beyond the Genome. 16-20 July 2000. Birmingham, UK

CA17 Carol Ryder, Adrian Sarjeant, Malcolm Bennett, Graham J. King (2000). Organisation & allelic analysis of the LAX gene family in *Brassica oleracea.* GAIT meeting, Nottingham.12th Apr 2000

CA18 Teakle GR, Kop EP, Smith LB, Ryder CD, McClenaghan ER, Barker G, Naylor RH, Patel D & King GJ (2001) Complexity and comparison of floral regulatory networks in Brassica and Arabidopsis. EMBO workshop: The Molecular Basis of the Floral Transition, JIC, Norwich.

CA19 Howell, EC, Ryder CD, Jones, G, Kearsey, M, King GJ, Armstrong S (2001) Physical mapping of chromosomes of *Brassica oleracea* var. alboglabra using fluorescence in situ hybridisation (FISH). Plant & Animal Genome IX Conference, San Diego

CA20 Barker GC, Naylor R, Patel, D, Grant, NJ, Ryder, CD, Howell, E, Beynon, JL & King, GJ. (2002). Advances in the construction of a physical map of *Brassica oleracea*. In: Proceedings of the Third GARNet Functional Genomics Meeting, 17-18 September 2002, York, UK, (ed K. Van de Sande)

CA21 Teakle GR, Kop EP, McClenaghan ER, Ryder CD, Smith LB & King GJ. (2002) Locus replication and the floral transition in Brassica. In: Procedings of the third GARNet Functional Genomics Meeting, 17-18 September 2002, York, UK, (ed Van de Sande)

CA22 Ryder CD, Barker GC, Howell, EC, Jones, GH, Kearsey, MJ, King GJ, Armstrong SJ (2002) Characterisation of an inverted, duplicated region of a *Brassica oleracea* chromosome. In: Procedings of the third GARNet Functional Genomics Meeting, 17-18 Sept. 2002, York, UK, (ed Van de Sande)

CA23 Barker GC, Naylor R, Patel D, Grant NJ, Ryder CD, Howell EC, Beynon JL & King GJ (2002). A comparative physical map of the replicated regions of *Brassica oleracea* genome with homology to a 3mb region of chromosome one of *Arabidopsis thaliana*. In: Investigating Gene Function Forum, July 2002

CA24 Barker GC, King GJ, Naylor R, Patel, D, Ryder CD, Bancroft, I, Beynon, JL, Kearsey, M & Scott R (2002). Development and comparative analysis of a *B. oleracea* physical map, anchored to the Arabidopsis genome. In: Proceedings of the Plant, Animal and Microbe Genome Conference, San Diego, January 2002, [no pp].

CA25 Barker GC, Patel D, Naylor R, Ryder C, Bancroft I, Kearsey M, Scott R, Trick M, Beynon J, King G. (2002) A comparative physical map of the

replicated regions of *Brassica oleracea* genome with homology to a 3Mb region of chromosome one of *Arabidopsis thaliana.* Plant & Animal Genome Conference X, San Diego. Jan 2002.

CA26 Barker GC, Naylor R, Grant NJ, Stevenson S, Ryder CD, Beynon JL & King GJ (2003) The physical map of the Brassica C genome: A valuable resource for the Brassica community. Procedings of the second Plant GEMs (and the fourth GARNet Functional Genomics) Meeting, 2-6 September 2003, York, UK.

CA27 Barker GC, Ryder CD, King GJ. Evidence for long-range sequence variation following duplication events within *Brassica oleracea.* International Polyploidy Conference, Linnean Society and Royal Botanic Gardens, Kew. 27-30 April 2003, London, UK.

CA28 Ryder CD, Barker GC, Howell EC, Jones GH, Kearsey MJ, King GJ, Armstrong SJ. Characterisation of an inverted, duplicated region of a *Brassica oleracea* chromosome. Plant & Animal Genome XI, San Diego Jan 11-15th 2003

CA29 M. Rousseau, O. Coriton, F. Eber, E. Jenczewski, B. Chalhoub, C. Ryder, G.Barker, G. King, A-M Chèvre. Identification of genome specific markers in oilseed rape. Joint meeting of the 14th Crucifer Genetics Workshop and the 4th ISHS Symposium on Brassicas. Oct 24th -28th 2004. Chungnam National University, Daejeon, Korea.

CA30 Guy Barker, Carol Ryder, Jay Moore, Rowena Naylor, Rachel Edwards, Andrew Sharpe, Jonathan Durkin, Derek Lydiate, Graham King. A public EST sequencing programme for *Brassica oleracea*. Joint meeting of the 14th Crucifer Genetics Workshop and the 4th ISHS Symposium on Brassicas. Oct 24th -28th 2004. Chungnam National University, Daejeon, Korea.

CA31 Guy Barker, Carol Ryder, Rowena Naylor, Rachel Edwards, Andrew Sharpe, Jonathan Durkin, Derek Lydiate, Graham King. A Public EST sequencing programme for *Brassica oleracea.* 5th Annual Genomic Arabidopsis Research Network (GARNet) and Brassica Research community, University of Leicester, 1st - 2nd September 2004.

CA32 Carol Ryder, Guy Barker, Neale Grant, Helen Robinson, Graham Teakle, Graham King. Use of Arabidopsis mutants to study the epigenetic regulation of curd formation. 5th Annual Genomic Arabidopsis Research Network (GARNet) and Brassica Research community, University of Leicester, 1st - 2nd September 2004.

CA33 Guy Barker, Carol Ryder, Rowena Naylor, Keith Edwards, Rachel Edwards, Graham King. Sequence comparison of three triplicated *Brassica oleracea* loci that share collinearity with the *AP1* locus of *Arabidopsis thaliana.* Plant & animal Genome XII, San Diego Jan 10-14th 2004.

CA34 Ksiazczyk, Tomasz, Ryder, Carol and Maluszynska Jolanta. The use of *Brassica oleracea* BAC clones in Physical Mapping of *Brassica campestris* and *Brassica napus* chromosomes. 8th Gatersleben Research Conference: Genetic Diversity & Genome Dynamics in Plants. 3rd-6th June, 2005.

CA35 Graham B Seymour, Carol D Ryder, Volkan Cevik, John Hammond, Alexandra Popovich, Graham J King, Julia Vrebalov, James J Giovannoni and Kenneth Manning. The MADS-RIN gene is a conserved ripening regulator in both non-climacteric and climacteric fruits. Gordon Conference - Postharvest Physiology, Connecticut College, New London, Connecticut. July 9-14, 2006

CA36 Karine ALIX, Carol D. RYDER, Jay MOORE, Graham J. KING and Pat HESLOP-HARRISON. The Diversity and Organization of Retrotransposons in *Brassica*: Understanding their Phylogeny and Evolution. Plant & animal Genome XIV, San Diego Jan 14th -18th, 2006

CA37 John Andrews, James R. Lynn, Nicholas Parsons, Carol D. Ryder, Philip J. White and Andrew J. Thompson. Genetic analysis of root traits for water capture in the genus *Solanum.* AAB conference - Resource Capture By Crops. Nottingham 10-12th Sept 2008.

CA38 Andrew Thompson, Carol Ryder, Howard Hilton, James Lynn, Graham Teakle, Dave Pink. Genetics of water use efficiency in *Brassica oleracea.* UK-BRC meeting, WHRI, Wellesbourne, 21st May 2008.

CA39 Pat Heslop-Harrison, Karine Alix, Johann Joets, Carol Ryder, Jay Moore, Guy Barker, Graham King, John P. Bailey. Brassica repetitive elements. UK-BRC meeting, WHRI, Wellesbourne, 21st May 2008.

CA40 Johann JOETS, Carol D. RYDER, Jay MOORE, John P. BAILEY, Graham J. KING, J. S. (Pat) HESLOP-HARRISON, and Karine ALIX. *In silico* characterisation of the *Brassica oleracea* genome-specific CACTA transposon *Bot1*. International Congress on Transposable Elements (ICTE), St Malo, France, April 20-23rd, 2008.

CA41 Karine ALIX, Johann JOETS, Carol D. RYDER, Jay MOORE, John P. BAILEY, Graham J. KING, and J. S. (Pat) HESLOP-HARRISON. The CACTA

transposon *Bot1*: genome-specificity and role in genome and gene evolution in *Brassica.* International Congress on Transposable Elements (ICTE), St Malo, France, April 20-23rd, 2008.

R01 King, GJ & Ryder CD (1996) Confidential report on work carried out for industrial contract: 'DNA assessment of self-incompatibility in *Brassica oleracea* L.'10pp.

R02 King, G.J, Brown, L., Ryder, C. & Periam, N. (1998) Microsatellite markers for accession identification, pedigree analysis and assessment of allelic diversity in Malus genetic resources. In. Report of a Working Group on Malus/Pyrus, Dublin 15-17 May 1997: 82-89. International Plant Genetic Resources Institute, Rome.

S01 25 retroelement & retroelement related sequences (2001). GenBank Accessions: AJ AJ414054- AJ414056, AJ414067-AJ414068, AJ414079-AJ414090, AJ421226-AJ421233

S02 23 retroelement & retroelement related sequences (2002) GenBank Accessions: AJ417791-AJ417813

S03 87 Rosaceae microsatellite sequences (2004) GenBank Accessions: Malus AY861467-AY861544 (78) Prunus AY861545-AY861553 (9) S04 3 *Brassica oleracea* whole BAC sequences (2008) GenBank Accessions: EU642504-EU642506

5.5 Citation data for AUTHOR'S published Works.

This data was obtained from the ISI web of knowledge. (http://wok.mimas.ac.uk) and was accessed on July 8th 2012. The emboldened entries are presented for examination in this covering document.

Publication details	Citation count
J. Brace, C.D. Ryder & D.J. Ockendon. (1994) Identification of S-alleles in *Brassica oleracea.* Euphytica. 80: pp229-234.	5
P.A. Roche, F.H. Alston, C.A. Maliepaard, K.M. Evans, R. Vrielink, F. Dunemann, T. Markussen, S. Tartarini, L.M. Brown, C. Ryder, G.J. King. (1997) RFLP and RAPD markers linked to the rosy leaf curling aphid resistance gene *(Sd_1)* in apple. Theoretical and Applied Genetics. 94: pp528-533.	36
C. Maliepaard, F.H. Alston, G. van Arkel, L.M. Brown, E. Chevreau, F. Dunemann, K.M. Evans, S. Gardiner, P. Guilford, A.W. van Heusden, J. Janse, F. Laurens, J.R. Lynn, A.G. Manganaris, A.P.M. den Nijs, N. Periam, E. Rikkerink, P. Roche, C. Ryder, S. Sansavini, H. Schmidt, S. Tartarini, J.J. Verhaegh, M. Vrielink-van Ginkel, G.J. King. (1998) Aligning male and female linkage maps of apple (Malus pumila Mill.) using multi-allelic markers. Theoretical & Applied Genetics. 97: pp60-73.	213
B. Sosinski, M. Gannavarapu, L.D. Hager, L.E. Beck, G.J. King, C.D. Ryder, S. Rajapakse, W.V. Baird, R.E. Ballard, A.G. Abbott. (2000) Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theoretical & Applied Genetics. 101(3): pp421-428	176
C.D. Ryder, L.B. Smith, G.R. Teakle & G.J. King. (2001) Contrasting genome organisation: Two regions of the *Brassica oleracea* genome compared with collinear regions of the *Arabidopsis thaliana* genome. Genome. 44: pp808-817	33
E.C. Howell, G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, E.P. Kop, C.D. Ryder, G.R. Teakle, J.G. Vicente, S.J. Armstrong. (2002) Integration of a cytogenetic map with a genetic linkage map of *Brassica oleracea.* Genetics. 161(3): pp1225-1234	53
R. Liebhard, L. Gianfranceschi, B. Koller, C.D. Ryder, R. Tarchini, E. van de Weg & C. Gessler. (2002) Development and characterisation of 140 new microsatellites in apple (Malus x domestica Borkh.). Molecular Breeding. 10(4): pp217-241	203
M.J. Aranzana, A. Pineda, P. Cosson, E. Dirlewanger, J. Ascasibar, G. Cipriani, C.D. Ryder, R. Testolin, A. Abbott, G.J. King, A.F. Iezzoni, and P. Arús. (2003) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theoretical & Applied Genetics. 106: pp819-825	127
E.C. Howell, G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, C.D. Ryder, S.J. Armstrong. (2005) Physical organization of the major duplication on *Brassica oleracea* Chromosome O6 revealed through fluorescence *in situ* hybridization with *Arabidopsis* and *Brassica* BAC probes. Genome. 48(6): pp1093-1103.	16

K. Alix, C.D. Ryder, G.J. King, J. Moore & J.S. Heslop-Harrison. (2005) The genomic organization of retrotransposons in *Brassica oleracea*. Plant Molecular Biology. 59(6): pp839-851	21
M. Leflon, F. Eber, J.C. Letanneur, L. Chelysheva, O. Coriton, V. Huteau, C.D. Ryder, G. Barker, E. Jenczewski and A.M. Chèvre. (2006) Pairing and recombination at meiosis of *Brassica rapa* (AA) x *Brassica napus* (AACC) hybrids. Theoretical & Applied Genetics. 113: pp1467-1480.	41
Stéphane D. Nicolas, Guillaume Le Mignon, Frédérique Eber, Olivier Coriton, Hervé Monod, Vanessa Clouet, Virginie Huteau, Antoine Lostanlen, Régine Delourme, Boulos Chaloub, Carol D. Ryder, Anne Marie Chèvre and Eric Jenczewski. (2007) Homoelogous recombination plays a major role in chromosome rearrangements that occur during meiosos of *Brassica napus* haploids. Genetics. 175: pp487-503.	38
Karine Alix, Johann Joets, Carol D. Ryder, Jay Moore, Guy Barker, John P. Bailey, Graham J. King & J.S. (Pat) Heslop-Harrison. (2008) The CACTA transposon *Bot1* played a major role in Brassica genome divergence and gene expansion. Plant Journal. 56(6): pp1030-44	16
Volkan Cevik, Carol D. Ryder, Alexandra Popovich, Kenneth Manning, Graham J. King and Graham B. Seymour. (2010) A FRUITFULL-like gene is associated with genetic variation for fruit flesh firmness in apple (Malus domestica Borkh.) Tree Genetics & Genomes. 6(2): pp271-279	4
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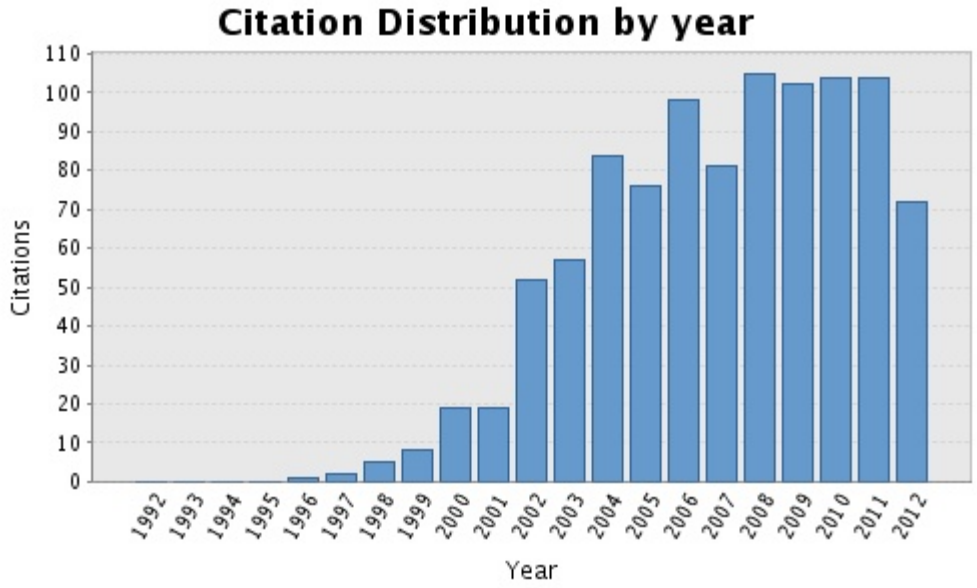
5.6 Citation Data for AUTHOR'S Peer Reviewed Published Works



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Average Citations per Article: **65.93**

h-index: **12**

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