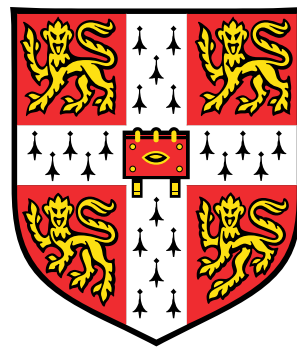


# Genetic and environmental regulation of olfactory sensory neurone diversity



**Ximena Ibarra Soria**

Wellcome Trust Sanger Institute

University of Cambridge

This dissertation is submitted for the degree of  
*Doctor of Philosophy*



To mom and James for being there every step of the way.



## **Declaration**

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other University. This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text. This dissertation contains fewer than 60,000 words excluding bibliography, figures and appendices, as per the requirements of the Degree Committee for the Faculty of Biology.

Ximena Ibarra Soria  
September 2015



## Acknowledgements

First and foremost, I would like to thank my supervisor, Darren Logan, for his continued support in and outside the lab. For always being available and willing to help; for his patience and encouragement. Very importantly, for teaching me how to think about science and for sharing all his wisdom and wit. There are a few people that have had a tremendous impact in my academic formation and, without a doubt, Darren is one of them; for that I will always be thankful.

I would also like to acknowledge all the people in the lab. Gabi who provided great advice and ideas to improve experiments, and was always happy to help. Luis, for being open to collaborate and combine the best of our abilities to create greater things. Maria, for working alongside me and making the lab a happier place. Elizabeth, Cristina and Sebastian for all their help with the wet lab. Laura and Sophia for useful discussions.

A big thank you to team 113, who made me an honorary member of their group. For all the lunch-time *insightful* discussions and laughter; and for including me in their dinners, Christmas celebrations, trips and outings. You have made my PhD a much happier one. Special thanks go to Daniela, who has always been there for me, sharing every step of the way. For her friendship and love, for the long talks over brunch, for the encouragement on the tough times and for always making sure I was ok.

I would also like to thank John Marioni, my secondary supervisor, for his help with the bioinformatics and for letting me pick his brains when I was struggling to understand my data. And to the rest of my thesis committee, David Adams and Gregory Jefferis who, along with John and Darren, provided very useful advice and comments on the progress of my research and how to make it better.

A lot of my PhD work was greatly facilitated by the staff of the Research Scientific Facility and the Illumina Bespoke Sequencing team. Special thanks to Mairi Kusma, Andrea Kirton and Nathalie Smerdon for invaluable help with many projects.

I owe big acknowledgment to my parents, for making sure I had the best opportunities to achieve whatever I wanted. To mom, for giving up so much so that I could have the best life possible; without your support I wouldn't be here. To my brother, for drawing

pretty mice I could use without infringing copyright, and for his support and advice along the way. Also, to Yuyi, who has always kept an eye on me even while being so far away.

A big thank you to James, for being along my side during this journey. For sharing his life with me and picking me up in the toughest times. For all his love and for making me a happier, better person.

Finally, I would like to acknowledge the Wellcome Trust for their generous support.



## Abstract

Animals use their sense of smell to gather plethora of information about their surroundings. The detection of odorants occurs in the main olfactory epithelium (MOE), which contains olfactory sensory neurones (OSNs) among other cell types; these express olfactory receptors (ORs) that bind to odorants. Each OSN expresses only one allele of one OR gene from a family of over 1,200 in the mouse genome. Thus, the mouse nose has over 1,200 different OSN types, each characterised by the OR expressed. High levels of genomic variation have been reported both in the mouse and human OR repertoire. This is thought to contribute to the unique sense of smell each individual has, but a large proportion of the observed phenotypic variance remains unaccounted for.

In this dissertation, I present the results from an RNAseq-based approach used to quantify the OSN repertoire of the mouse. Firstly, I validated the accuracy and reproducibility of this technology to study the olfactory system. I then characterised the transcriptome of the MOE and of the OSNs as a population and at the single-cell level. This allowed me to conclusively prove that OR expression is indeed monogenic and monoallelic. Then, I demonstrated that the method is sensitive enough to detect the expression of almost the complete OR repertoire. Also, I was able to annotate full-length gene models for many OR genes.

Secondly, I explored the diversity of OSN types in three inbred strains of mice (C57BL/6, CAST/EiJ and 129S5) via their OR gene expression levels. I found that each strain has a unique and reproducible distribution of OSNs in their noses, and that genomic variation instructs this neuronal variance in *cis*. Finally, I analysed the plasticity of the distribution of the different classes of OSNs by stimulating animals with particular odorants. Exposure to an enriched olfactory environment results in the differential expression of dozens of OR genes in a reproducible and specific manner. These changes increase with time and are reversible. These data allow to comprehensively explore and dissect the effects of genetic and environmental variation on the regulation of OR expression and OSN repertoire. Together they generate an olfactory sensory system that is individually unique.



# Contents

Contents	xii
List of Figures	xv
List of Tables	xix
<b>1 Introduction</b>	<b>1</b>
1.1 The mammalian olfactory system. . . . .	1
1.1.1 The main olfactory epithelium. . . . .	3
1.1.2 The vomeronasal organ. . . . .	24
1.1.3 The septal organ. . . . .	31
1.1.4 The Grueneberg ganglion. . . . .	32
1.2 Regulation of OR expression. . . . .	33
1.2.1 <i>Cis</i> -acting elements influence OR expression. . . . .	37
1.2.2 Early-bird-gets-the-worm paradigm of OR expression. . . . .	39
1.2.3 Negative feedback ensures singularity. . . . .	43
1.3 Detection of odorants by olfactory receptors. . . . .	46
1.3.1 Combinatorial olfactory coding. . . . .	47
1.3.2 Deorphanisation of olfactory receptors. . . . .	52
1.3.3 Antagonism. . . . .	57
1.3.4 Adaptation and desensitisation of olfactory sensory neurones. . . . .	58
1.3.5 From detection to perception: impact of functional variation. . . . .	60
1.4 Plasticity of the olfactory system. . . . .	64
<b>2 The transcriptome of the mouse olfactory system.</b>	<b>71</b>
2.1 Transcriptome profiling by RNAseq. . . . .	74
2.1.1 Comparison to alternative methodologies. . . . .	77
2.2 Expression of the receptor repertoire. . . . .	79

2.2.1	Comparison to other methodologies. . . . .	83
2.2.2	Sensitivity of RNAseq to detect lowly expressed receptor genes. . . . .	84
2.2.3	The multiread problem. . . . .	84
2.2.4	Complete annotation of the gene models. . . . .	88
2.3	Identification of novel genes. . . . .	92
<b>3</b>	<b>Decomposing the WOM: from tissue to single-cell.</b>	<b>95</b>
3.1	The transcriptome of the olfactory sensory neurones. . . . .	96
3.2	Mature OSNs segregate into two distinct populations. . . . .	99
3.3	RNAseq of single OSNs. . . . .	101
3.3.1	Heterogeneity between single OSNs. . . . .	105
3.3.2	Monogenic expression of OR genes. . . . .	107
3.3.3	Monoallelic expression of OR genes. . . . .	110
3.3.4	Identification of a novel type of OSN. . . . .	111
<b>4</b>	<b>Genetic variation and the expression of the OR repertoire.</b>	<b>115</b>
4.1	Gender has little effect on OR gene expression. . . . .	116
4.2	Some OSN types are more abundant than others. . . . .	117
4.3	OR expression differs between mouse strains. . . . .	119
4.4	The genetic background determines OR expression levels independent of odour environment. . . . .	125
4.5	OR expression is controlled in <i>cis</i> . . . . .	129
<b>5</b>	<b>Olfactory stimulation alters the OR repertoire.</b>	<b>133</b>
5.1	Acute but not chronic odour exposure affects OR expression levels in the WOM. . . . .	134
5.2	Differential regulation of OR genes is odour-specific. . . . .	137
<b>6</b>	<b>Discussion and future perspectives</b>	<b>141</b>
6.1	Understanding the mouse olfactory system by RNAseq. . . . .	142
6.2	Almost all OR genes are expressed in the MOE. . . . .	147
6.3	The MOE is a mosaic of OSN types. . . . .	148
6.4	Plastic control of OSN diversity. . . . .	152
6.5	Functional impact of differences in OSN number. . . . .	154
	<b>References</b>	<b>157</b>

---

<b>A</b>	<b>Methods</b>	<b>189</b>
<b>B</b>	<b>Supplementary tables</b>	<b>199</b>
<b>C</b>	<b>Papers produced during my PhD.</b>	<b>209</b>
C.1	Papers associated with this dissertation. . . . .	209
C.2	Other papers. . . . .	209



# List of Figures

1.1	The mammalian olfactory system . . . . .	2
1.2	Composition of the MOE . . . . .	3
1.3	Turbinate structure of the MOE . . . . .	4
1.4	Olfactory signal transduction cascade . . . . .	8
1.5	The mouse olfactory receptor gene family . . . . .	11
1.6	Olfactory receptors are expressed in zones . . . . .	14
1.7	OSN axons coalesce into glomeruli . . . . .	16
1.8	Glomerular organisation in the olfactory bulb . . . . .	18
1.9	The mouse vomeronasal organ . . . . .	25
1.10	Signal transduction proteins in vomeronasal sensory neurones . . . . .	26
1.11	The mouse vomeronasal receptor gene family . . . . .	29
1.12	A feedback mechanism ensures singular OR expression . . . . .	45
1.13	Combinatorial odour coding . . . . .	47
1.14	Molecular range of ligands for the mOR-EG and mOR-EV receptors . . . . .	50
1.15	<i>In vitro</i> expression of ORs in Hana3A cells . . . . .	56
1.16	Adaptation of OSNs to repeated stimulation . . . . .	59
1.17	Individualised OR repertoire leads to unique perception . . . . .	63
2.1	Correlation between biological replicates . . . . .	75
2.2	The transcriptome shows a bimodal distribution of low- and high-expressed genes . . . . .	76
2.3	The transcriptome of the VNO and the WOM . . . . .	77
2.4	Comparison of the RNAseq expression values versus the microarray intensity data . . . . .	78
2.5	Comparison with qRT-PCR TaqMan expression assays . . . . .	79
2.6	Expression of the VR repertoire . . . . .	81
2.7	Expression of the OR repertoire . . . . .	81

2.8	Genes are expressed higher than pseudogenes . . . . .	82
2.9	Comparison of the receptor expression across different platforms . . . . .	83
2.10	Expression of a deleted OR cluster . . . . .	85
2.11	Uniqueness of the receptor sequences . . . . .	86
2.12	Multireads mapped to VR and OR genes . . . . .	87
2.13	Full length gene models for OR and VR genes . . . . .	88
2.14	Number of transcripts per receptor gene . . . . .	89
2.15	Improved receptor gene models are more unique . . . . .	90
2.16	Expression of the receptors with the new gene models . . . . .	91
2.17	Novel genes expressed in the olfactory system . . . . .	93
3.1	Differentially expressed genes between the OSNs and WOM . . . . .	97
3.2	Comparison to Sammeta et al. . . . .	98
3.3	Receptor expression in the WOM vs the sorted OSNs . . . . .	98
3.4	FACS plot of OMP-GFP animals . . . . .	100
3.5	GFP <sup>+</sup> neurones are mature . . . . .	100
3.6	Differentially expressed genes between the GFP <sup>low</sup> and the GFP <sup>high</sup> cells . . . . .	101
3.7	Quality control of the single-cell RNAseq data . . . . .	102
3.8	Correlation of single OSNs to other datasets . . . . .	104
3.9	Expression of canonical markers . . . . .	105
3.10	Correlation between two single OSNs . . . . .	106
3.11	Highly variable genes pattern single OSNs . . . . .	107
3.12	OR expression in single OSNs . . . . .	108
3.13	Monogenic expression of OR genes . . . . .	109
3.14	OR expression in several single-cell datasets . . . . .	110
3.15	OR expression is monoallelic . . . . .	111
3.16	Characteristic expression profile of a novel type of OSN . . . . .	112
3.17	Validation of the DE genes in no-OR cells . . . . .	113
4.1	Transcriptome of the WOM of males and females . . . . .	116
4.2	OR expression in males and females . . . . .	117
4.3	RNAseq expression correlates with neurone number . . . . .	118
4.4	Effect of imputing genomic variation on OR expression estimates . . . . .	119
4.5	Normalisation for OSN number . . . . .	120
4.6	OR expression in B6 and 129 . . . . .	121
4.7	OR expression in B6 and CAST . . . . .	122



---

4.8	Differential expression of the OR repertoire in three strains of mice . . .	123
4.9	Differentially expressed OR genes are more variable . . . . .	124
4.10	Expression of a polymorphic pseudogene . . . . .	124
4.11	Differentially expressed OR genes are clustered in the genome . . . . .	125
4.12	Experimental design to dissect genetics from environment . . . . .	126
4.13	OR expression is determined by the genetic background . . . . .	127
4.14	OR expression in B6 pups . . . . .	128
4.15	Pseudogene OR expression in B6 . . . . .	129
4.16	OR expression is regulated in <i>cis</i> . . . . .	130
5.1	Odour exposure experimental set-up . . . . .	134
5.2	OR expression is altered with acute stimulation . . . . .	135
5.3	DE OR genes in acutely exposed mice . . . . .	136
5.4	ORs regulated by odour stimulation change in a time-dependent manner	136
5.5	Changes in OR abundance are plastic . . . . .	138
5.6	OR genes respond to odour stimulation in a specific manner . . . . .	138
5.7	DE OR genes in mice stimulated with different odorants . . . . .	139
5.8	Different ORs respond to specific odorants . . . . .	140
6.1	Genetic and environmental regulation of OSN diversity . . . . .	143



# List of Tables

2.1	Putative novel genes . . . . .	92
B.1	Sequenced samples presented in this dissertation . . . . .	203
B.2	Mapping statistics of RNAseq samples . . . . .	206
B.3	VR genes not properly annotated in Ensembl . . . . .	207
B.4	Mapping statistics of RNAseq single-OSN samples . . . . .	208