# The ecology and anatomy of scent

# in the critically endangered kakapo (*Strigops habroptilus*)

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Hoki, the kakapo - Photo by Dr. Luis Ortiz Catedral

Kakapo chicks born in 2008;©Photo by Chris Birmingham

# ABSTRACT

The focus of the research presented here is the analysis of feather scent emitted by a parrot, the kakapo (*Strigops habroptilus*) and the kakapo's ability to perceive scent by studying the anatomy of its brain and the olfactory bulb. In addition, behavioural research was conducted to determine the capability of the kakapo's closest relatives, the kea (*Nestor notabilis*) and kaka *(N. meridionalis*) to detect scents and to distinguish between different concentrations of scents.

The strong odour of the kakapo is one of the many unique characteristics of this critically endangered parrot, but its sense of smell has never been described in detail. The kakapo is the largest parrot worldwide, it is nocturnal and flightless. Kakapo are herbivorous and it is the only parrot with a lek breeding system. Males defend several display arenas during the breeding season and continuously produce low frequency booming calls. Females come from afar and appraise different males and choose one with which they want to mate. As in all lek mating systems some males make major contributions to the gene-pool of the next generation while others make little or no contribution. Currently it is not known what the female's choice is based on and why some male kakapo are 'favoured' over others. However, it has been observed that favoured males appear to emit a stronger odour than less attractive ones (pers. comm. Kakapo Recovery Team). This study is the first to compare the chemical composition of the kakapo's scent in relation to season, age and sex. It is also the first study to uncover the kakapo's ability to smell by conducting a comparative examination of the anatomy and histology of the brain and the olfactory bulb.

In spite of its endangered status, the kakapo is a good model in which to study olfaction, as the birds are closely monitored by the Department of Conservation, New Zealand. The birds undergo regular health checks and transmitter changes, allowing access to a large proportion of the population at once and for which their individual history is known. The study of olfaction in kakapo is important as it can contribute to the growing field of avian olfaction, and by elucidating the kakapo's potential for olfaction conservation managers will be able to make better decisions in their attempt to save this species. The research approach adopted in this dissertation includes the analysis of feather samples from individuals of different sex and age as well as from different seasons using gas chromatography-mass spectrometry. The opportunity to examine the brain as well as the eyes of a kakapo that died at Auckland Zoo, Auckland, New Zealand, allowed a comparative study of the brain, the olfactory bulb and the visual centres (of both the thalomofulgal and the tectogucal pathways) with other Australasian parrots. Additionally, behavioural experiments with kea and kaka, the closest relatives

of the kakapo, give insight into two of New Zealand endemic parrot's and their ability to distinguish between different scents and scents of different concentrations.

The findings from this research provide evidence that kakapo distinguish themselves by having one of the largest olfactory bulbs measured in parrots and the highest number of mitral cells, responsible for the transmission of an olfactory neural signal into a behavioural response, counted in any species to date. They also have a strong odour, whose chemical composition shows sexual, age-dependent and seasonal distinction. Furthermore, the study found that kea and kaka are both able to distinguish between different scents and different concentrations of scents.

The main conclusions drawn from this study are that kakapo appear to be equipped with a functional olfactory bulb, able to sense olfactory information, but also communicate information that is likely to be of social importance using their plumage scent. In conclusion, this dissertation provides the foundation for future research, in particular to examine the role of the scent in the social life in kakapo, and it provides fundamental insight into the olfactory and visual sensory abilities of the New Zealand endemic kakapo.


Rebecca Wu

# REFLECTION AND ACKNOWLEDGEMENTS

I guess my mother Heidemarie would have been fascinated by the many stories I can tell about kakapo, a bird so curious it seems to originate from a different world. She had an eye for detail and taught my sister and myself to look at things with the eye of an eagle and to appreciate them. I started collecting feathers when I was seven years old. I had a big cardboard box with Indian red patterns on its side, which held all the treasured feathers found on many excursions to various west European woods and the Swiss Alps. It was greatly improved by my early voluntary work in the zoo, which meant that I had access to the most exotic feathers. They were all stored in this box, and I was welcomed with a nice wooden, earthy smell whenever I opened the box.

Little did I know at that time how important feathers would become later in my life. I still collect them, but not only for pleasure anymore. Before that though, my journey took me to Africa - inspired by my grandfather who had worked as a medical doctor in Ifakara, Tanzania, in his early days and who wanted to see this amazing continent for one last time. He invited the whole family to a memorable trip to Zambia, where my wish to work in Africa was born. This wish was fulfilled when I conducted by MSc in Tim Clutton-Brock's unique meerkat research project in the Kalahari and worked on aspects of the rich variation of marking behaviour in the social mongoose, the meerkat, (*Suricata suricatta*). I was familiarised in a vivid way with the many ways scent and marks can be transferred in animals. Work in the Botswana Wild Dog Research Project on the African wild dog (*Lycaon pictus*) to develop a biological method to repel wild dogs from farmland taught me the conservational aspect scent can have. A call to New Zealand to work on olfaction in the Northern brown kiwi (*Apteryx mantelli*) heralded a new and fascinating period in my life. Hitherto, little was known about olfaction in birds and the offer to work on the Northern brown kiwi was all the more tempting since I had to deal with a bird whose behaviour, in many aspects, is more reminiscent of a mammal than a bird. Preliminary behavioural experiments undertaken on free ranging birds indicated the use of body scent in the social life of kiwi and a pilot analysis of the chemical compounds in body scent using gas chromatography-mass *spectrometry* revealed the presence of aromatic oils and alcohols that could account for the strong smell of this bird (Castro *et al*. 2010). Periods of waiting for permits were filled with work on rats, with the aim to trial and use the scent of conspecifics to attract and lure them. In a land where food is plentiful, the attraction to mate might be stronger and serve as a good option to lure and catch rats while presenting a low risk to other wildlife. All this work on diverse animals using scent and olfaction was a good introduction for my PhD on another endangered and strong smelling bird, the kakapo (*Strigops habroptilus*), surely the most bizarre and controversial, but rewarding, animal I have worked on so far.

All these ventures to far away destinations were supported by my father Hans-Otto, who in return got to see all these exciting places. I am indebted with deep thanks and gratitude to my father for having supported all along his daughter's unusual, extensive, and not always easy, travel destinations in many ways. I am not quite sure how I came to land so far way, but I think we can blame the kakapo.

I am greatly thankful to my sister Alena Sonja for being all ears to all sorts of issues that come along in the course of a PhD. I would like to thank her for proofreading and commenting on some aspects of my work. Let alone the countless care parcels of food she and my father supplied, primarily fine Swiss and Belgian chocolate, as well as the supply of Italian coffee and other specialities, bringing home a little closer. I think we kept MAF quite busy over the last few years.

Deepest and sincere thanks go to my partner David Vincent, who supported me in the best possible way. He encouraged me in the first place to return to New Zealand in order to study New Zealand's smelly avian fauna, which I am more than thankful for. I would also like to thank David for proofreading some of my work. Coming from a very different background, his knowledge in economy brought me a different perspective to my life. I am also thankful for that reason.

Apart from all the personal support that has been given to me by my partner and my family, this thesis could not have been completed without the continuous and never failing support of my supervisor Assoc. Prof. Dr. Dianne Brunton (Director of the Ecology and Conservation Group, Institute of Natural Sciences, Albany Campus, Massey University, New Zealand). Dianne has all the aspects that a student wishes for in a supervisor, and I am thankful for her openness to new research projects, for sharing the vision and supporting and refining ideas and helping to bring them to their successful completion. Dianne makes time for her students even if she has no time, and lives the Kiwi 'can do' attitude to the fullest. Without her input and help, this project would not have been possible.

The second person in my academic field I am indebted to is Dr. Ron Moorhouse (Scientist, Kakapo Recovery Programme, Invercargill, New Zealand). Not only am I thankful for his support and help with pursuing an initially unusual project idea and helping with the permits, but also for being a marvellous source of knowledge and providing me with deep insights into the wondrous world of the kakapo.

My co-supervisor Dr. Weihong Ji knew Ron Moorhouse personally and not only enabled me to get to know Ron from a different perspective, that is as a marvellous Scottish pipe player, but made the first contact possible. I am grateful for Dr. Weihong Ji's support and help.

There were several large groups with which I worked on my exciting subject. One of the most remarkable groups, however, was my 'GC-MS group', filled with people that were just as much in love with the subject as I am, and always eager to know more – no matter how late at night it was. So it was more than natural to share knowledge and to keep each other up to date with the newest information available. A vibrant, high wired exchange of knowledge took place between my co-supervisors Prof. Tom Goodwin (Hendrix College, Conway, Arkansas, USA) and Assoc. Prof. Dr. Julie Hagelin (Institute of Arctic Biology, University of *Alaska,* Fairbanks, Alaska, USA) and my collaborators Prof. Dave Greenwood (The School of Biological Sciences, Auckland University, Auckland, New Zealand) and Dr. Andrew Fidler (Cawthron Institute, Nelson, New Zealand). I would like to thank Dave for his tremendous help with the gas chromatographic work and evaluation of the GC-MS output, thank you for putting endless hours into this work, but it has all been worthwhile! I would like to thank Julie and Andrew as well as Pat Holland (Cawthron Institute, Nelson, New Zealand) at this point for allowing me to continue their exciting journey in olfaction in kakapo. Their initial results opened the way for this thesis and helped it to success. I think we all were highly inspired and carried away by Tom's unforgettable humour. It has been great pleasure to work with you people and Tom's visits to New Zealand (Figure 1) are just as vivid as our (Tom, Dave, Julie and myself) trip to the Chemical Signals in *Vertebrates* Conference in *Berlin,* Germany, August, 2011. Thank you for being there, and I truly hope, we will continue our adventure in the world of scent in kakapo.

**Figure 1** Dianne (left), Tom (middle) and Andrew (right) on a work lunch meeting that we enjoyed close to Massey University, Albany, New Zealand.

Insight, knowledge and an understanding of not only working with brains but getting a flair for understanding their anatomy, were all facilitated with a lot of patience and understanding by Dr. Jeremy Corfield (Department of Neuroscience, *University of Lethbridge, Lethbridge*, Alberta*, Canada*). Without his unlimited help and time, I would never have come to the point of anatomical understanding that I gained in my work. I am deeply indebted to profound and sincere thanks for all the advice, help and understanding Jeremy has given me.

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I had the pleasure of examining the olfactory abilities of kea and kaka during my PhD. I am grateful to Auckland Zoo for permitting me to work in their native fauna section. In particular, I would like to thank Andrew Nelson, Michelle Whybrow, Natalie Clark, Tanya Shennan, Debs Searchfield, Sarah Anderson, Helen Watson and Claire Cameron for helping with the experiment.
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**'Nothing is by chance'**

Eileen Caddy

# THESIS STRUCTURE AND FORMAT

This thesis is written as a series of seven interrelated chapters, two of which (Chapters Five and Six) have been published in part in peer reviewed journals (Corfield *et al*. 2011; Gsell *et al*. 2012). ***Chapter One: scent and olfaction in birds; kakapo (Strigops habroptilus) as a model species*** introduces the main subject of my thesis by discussing the current knowledge on olfactory signalling in birds and reviewing the literature on the use of olfaction in birds. The particular case of the kakapo is reviewed by addressing its history and evolution, as well as its behavioural characteristics and the current conservation status. Additionally, it is described why the kakapo makes an excellent model in which to study olfaction in birds. At the end of this first chapter, a brief summary of the specific aims of this thesis is presented.

Chapters Two to Six are data chapters and can be divided into four sections. The first section, Chapter Two, deals with the characteristics of the scents emitted. The second section, Chapter Three and Four, deals with the anatomical specialization for scent perception, the brain and the olfactory bulb, in particular. The third section, Chapter Five, looks at the visual system in kakapo, in order to evaluate the importance of olfactory versus visual cues. The fourth section, Chapter Six, combines both aspects of emitting and perceiving scent and looks at whether the kakapo closest relatives, the kea (*Nestor notabilis)* and the kaka (*N. meridionalis)* possess olfactory abilities by using behavioural experiments. Chapter Seven consolidates all findings.

***Chapter Two: The chemical analysis of kakapo (Strigops habroptilus) feather scent.***

The strong, sweet smell of the kakapo has been described on many occasions, yet it is not known what role it plays. Regular health checks and transmitter changes in the remaining kakapo population on Codfish Island, New Zealand, provided me with the opportunity to obtain feather samples from different kakapo individuals, of different age and sex and collected at different seasons. This allowed me to conduct a complete analysis of the chemical composition of the feather odour in kakapo, encompassing age related, sexual and seasonal factors. Equipped with that data, I was able to assess what information kakapo can convey through its body odour. While Chapter Two discusses the qualities of the sweet smell of the kakapo and examines what type of information the kakapo is able to convey with the smell of its plumage, Chapters Three and Four address the ability of kakapo to receive and process olfactory information.

***Chapter Three: A comparison of brain stuctures of the nocturnal kakapo (Strigops habroptilus) and the diurnal sulphur-crested cockatoo (Cacatua galerita) with special emphasis on the olfactory bulb and the optic lobe :*** The rare opportunity to obtain the brain of an old, male kakapo that had died at Auckland Zoo, presented the unique opportunity to look at the general brain anatomy of the kakapo and to compare it with that of the diurnal sulphur-crested cockatoo.

***Chapter Four: Anatomy and histology of the olfactory bulb of the kakapo (Strigops habroptilus) in comparison to other Australasian parrots:*** Thanks to collaborators in Australia and Canada, I was able to examine and compare the detailed anatomy of the olfactory bulb of the kakapo and nine Australasian parrots of different behavioural ecology and size. These were the Australian king parrot (*Alisterus scapularis*), the cockatiel (*Nymphicus hollandicus*), the crimson rosella (*Platycercus elegans*), the Eastern ground parrot (*Pezoporus wallicus*), the Eastern rosella (*Platycercus eximius*), the galah (*Cacatua roseicapilla*), the rainbow lorikeet (*Trichoglossus haematodus*), the red-rumped parrot (*Psephotus haematonotus*) and the sulphur-crested cockatoo (*Cacatua galerita*). A detailed and comparative study of the anatomy and histology of the olfactory bulb in the kakapo, allowed me to address questions such as whether the kakapo has an acute sense of smell.

***Chapter Five: Anatomy and histology of the visual system of the kakapo (Strigops habroptilus) in comparison to other birds:*** An environment is always perceived through a variety of senses, although some sensory systems are more developed than others. Therefore, the general findings regarding the olfactory system of different bird species were compared to the development and the character of the visual systems, with particular reference to the specific situation in the kakapo.
The visual system was assessed in two ways because retinal information is conveyed over two major pathways: the thalamofugal pathway and the tectofugal pathway. In order to assess visual abilities, it is therefore important to examine the retina and specific brain compartments. For that reason, I describe the retina of the kakapo, while comparing it to the retina of other typical diurnal birds (the domestic chicken, *Gallus gallus*, and the rock pigeon, *Columba livia*), and nocturnal birds (the barn owl, *Tyto alba*, and the predominantly nocturnal morepork, *Ninox novaeseelandiae*). Additionally, I compared four visual brain centres (the entopallium, the nucleus rotundus, the tectum opticum and the Wulst) among nine different parrots. I contrasted the visual brain centres in the kakapo brain with those of the parrots used in the comparison made in the olfactory bulb. Only the Eastern ground parrot had to be replaced with a sample from the kea (*Nestor notabilis*), as the preservation quality of the brains did not always allow me to use them for all examinations. Parts of this work have been published in a peer-reviewed journal (Corfield *et al*. 2011 and Appendix A, Figure A1). Dr.

Jeremy Corfield gained first authorship in this paper due to his connections to Dr. Andrew Iwaniuk and for making this paper possible. Laboratory work has been equally conducted by myself and Jeremy Corfield, data analysis for the paper was mainly conducted by Dr. Jeremy Corfield and Dr. Andrew Iwaniuk, while write up was predominantly done by myself, Dr. Jeremy Corfield and Dr. Andrew Iwaniuk. The data presented in Chapter Five differs from what is presented in the paper, because I conducted my calculations with a different set of parrot species.

***Chapter Six: Olfactory sensitivity in kea and kaka:*** In order to evaluate whether the sense of smell and the action of scenting play any role in the ecology of the Nestoridae, I conducted scent experiments with the kakapo's closest relatives, the kea (*Nestor notabilis)* and the kaka (*N. meridionalis)* at Auckland Zoo. The experiments tested whether kea and kaka possess olfactory abilities and whether they are able to discern different scents and different concentrations of scents. The work presented in this chapter has been published in a peer-reviewed journal (Gsell *et al*. 2012 and Appendix B, Figure B1). I conducted all the experiments, the statistical analysis and the write-up, while my co-authors provided useful input.

***Chapter Seven: Conclusions and outlook:*** The last chapter summarises all information and puts it into context. The relevance of my findings is discussed and research directions are suggested.

***Appendixes A&B:*** Statement of contribution for a publication

***Appendixes C-H:*** present supporting information to Chapter Two.

***References:*** All references are listed at the end of the thesis to minimise repetition. All literature cited is consistent with the format used for the scientific journal: *Proceedings of the Royal Society, Sciences B*. For a list of title word abbreviations, see:
http://www.csa.com/ids70/serials_source_list.php?db=biolclust-set-c.

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