Divergence of a mammal along a habitat gradient:

A study of the coppery brushtail possum, *Trichosurus vulpecula johnsonii*.

Thesis submitted by

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STATEMENT OF SOURCES

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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STATEMENT ON THE CONTRIBUTION OF OTHERS

Financial Support

Financial support for this research was provided by James Cook University through IRA and Graduate Research School funding, and through a grant awarded by The Australia and Pacific Science Foundation. I was financially supported by an Australian Postgraduate Award stipend paid by the Australian Government.

Samples

Sam Price-Rees, with assistance from Katie Jones, captured 23 adult grey brushtail possums, two grey pouch-young, one coppery brushtail possum, and sampled three road kill possums for this study in 2005. The morphological measurements and genetic samples were collected for, and used in this study. Associate Professor John Winter contributed measurements and DNA samples from eight grey brushtail possums. One of these samples was not entered into this study as it was the pouch young of a female captured: juvenile measurements and the bias of including known offspring in a population genetics analysis meant that it could not be included in this study. Jane DeGabriel captured six coppery brushtail possums from the Atherton Tablelands for separate study, and contributed DNA and measurements from these animals to this study also.

Advice on methods

Associate Professor Steve Williams provided key recommendations on the field techniques and applications of distance sampling. He and Associate Professor John Winter also provided valuable advice regarding the distribution of brushtail possums along the eastern slopes of the Atherton Tablelands. Professor John Endler was instrumental in educating me on how to best perform colour analysis of fur photographs. Dr. Will Edwards provided advice on several statistical analyses and computer programs to utilize. Anna Pintor taught me how to run quantile regressions using my microsatellite relatedness/geographic distance data.

Supervisors

My four supervisors, Associate Professor Andrew Krockenberger, Associate Professor Brad Congdon, Professor Chris Johnson, and Professor Ross Crozier, together acquired the Australia and Pacific Science Foundation grant for this research, and provided their assistance and advice according to their different specialties in resolving the various difficulties and complexities that arose regarding the planning, implementation, analysis and reporting of this research.

Fieldwork

Many volunteers were instrumental in assisting the detection and capture of brushtail possums around the Atherton Tablelands: Steve Ryan, Anthony Mann, Westen Thomas, Luke, Peter Byrnes, Mel Commerford, Ilona and Pieter Moerman, Denise and Gareth, Sarah Schapel and William Hancock, Andrew Picone, Emma King, Louise Halritchie, Mandy Soymonoff, Martine Adriaansen, Madeline Ford, Tim Johns, Sarah Meyer, Natsumi Morita, Eri and Yuriko, Cherie Dugal. Russel Edwards, Brigitte and Georgina Humphries, Beau and Amy, Emma and Rick, Mel, Les and Mickey, each residents of Millaa Millaa, not only assisted my attempts to capture brushtail possums, but also very kindly allowed me to allow me to access their properties and to trap around their land in these endeavours.

Andrew Dennis, Paul Chiari and Kathy East also allowed access to their properties, provided advice about the preferences of local brushtails, and guided me around the local landscape so that I could access various established tracks and evaluate where to trap.

Michael Joyce and Pat Nagellega from QPWS were extremely helpful after cyclone Larry in helping me to access Herberton State Forest.

Johan Larson and Gabriel Porolak from James Cook University's Vertebrate Ecophysiology Laboratory volunteered their time in the field on numerous occasions, assisting in the capture of many brushtails.

DECLARATION ON ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human (*1999*),* the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997)*,* the *James Cook University Policy on Experimentation Ethics*. *Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval numbers A856, A1261 and A1262).

Signature: _____ Date:_____

Sarah Emily Kerr

ACKNOWLEDGEMENTS

My rainforest adventure began three and a half years ago. It has had its ups and downs, and a steep learning curve, as I suspect most PhD projects do, but through the assistance and encouragement of the following amazing people, it has been an immensely rewarding experience, and I thank them all very much for all their help.

I have been fortunate to have had the guidance of four brilliant and encouraging supervisors, each with different, complementary specialties. Together they acquired a grant from the Australia and Pacific Science Foundation, an organization to which I extend considerable thanks for their financial support of this research. Associate Professor Andrew Krockenberger, my principle supervisor, was instrumental in providing advice on rainforest field methods; teaching me how to shoot and tranquilize possums, climb trees, and combining the two, demonstrating how to very quickly collect a possum from eight meters up a tree when it has fallen asleep hanging by just the very tip of its tail! I thank Andrew also for sharing his time and expertise throughout this project; in planning, fieldwork, data analysis, seminar preparations and thesis writing; he has been an amazing mentor throughout my time as a PhD student, and I feel very fortunate to have had his guidance. Also based in Cairns, Associate Professor Brad Congdon provided invaluable advice about genetics techniques and theory. Brad's generosity with his time in explaining many of the more advanced or technical genetic concepts is something for which I am immensely grateful, and was particularly important in the successful design of MC1R colour gene primers, in carefully exploring the genetic structure of Atherton Tablelands brushtail possums from many different perspectives, and assisted my ability to later explain all that work here. Professor Chris Johnson was instrumental in my undertaking this project, and I thank him very much for this opportunity, and for his time and advice throughout this study, particularly regarding evolutionary processes, brushtail behavior, statistics, and thesis write-up. Professor Ross Crozier guided me into the world of phylogenetic analysis, shared with me his passion for both the history and the potential of genetic research (conversations

that often led to us climbing his rather tall book cases looking for some ancient algorithm or vertebrate-specific text), he introduced me to Genbank and the delights of simple curiosity in this realm, and with his enthusiasm, debate, humour and patience, Ross helped me to develop a far greater set of skills both in the lab and away from it. I miss his guidance immensely. Specific to this project, Ross was instrumental in sharing his time and expertise to help me examine and interpret the genetic data obtained, proof-read thesis drafts, provided advice regarding phylogenetic analyses, and guided me through the maze of genetic analysis software and the pitfalls that can arise without checking the assumptions within the algorithms. I consider it a great privilege and myself incredibly fortunate to have had Ross as my supervisor, and it saddens me deeply to think that never again will we climb his bookcases searching for a key paper, because Ross's office is now empty, and that there will be no more conversations between us of possums, of genetics, insects, music, elegant algorithms, or historical events, because he is gone. Yet I am heartened by the idea that having been such an important mentor to not just myself but a very large array of students, colleagues, friends and family, Ross's legacy will be enduring.

I was very fortunate to receive guidance from Associate Professor Steve Williams about how to conduct distance sampling surveys. Steve and Associate Professor John Winter also gave me some extremely timely advice about the distribution of brushtail possums around the eastern edge of the Atherton Tablelands; this undoubtedly saved me many long nights of persistent spotlighting for possums that weren't there. Professor John Endler gave me some important advice about analyzing colour from fur photographs, and I thank him very much for sharing his time and expertise. Dr. Will Edwards provided assistance with several statistical analyses. In particular, with the help of Will, and of Anna Pintor, I was introduced to the wonderful possibilities of quantile regressions, and I thank them both for their help, time and advice.

I am immensely grateful to several people who have donated brushtail samples to this project. Sam Price-Rees began the trapping for this project in 2005, and trapped most of the grey brushtail possums along the Herberton State Forest

transect. I thank Sam very much for not only her work in the field trapping the grey brushtails, but in scouting the Tablelands for potential sites, and together with Katie Jones, for introducing me to the region when I began my PhD, sharing tips about life at James Cook Uni., rainforest fieldwork, possums, and finding North Queensland's best curry and kebabs in Atherton!

Associate Professor John Winter generously provided me with eight grey brushtail possum DNA samples, and lent his time and encouragement on several occasions, with useful tips and advice about brushtail research in the Wet Tropics. I thank John very much for sharing his time, support and expertise over the course of this project.

Jane DeGabriel was conducting research at James Cook University's Townsville campus, studying the dietary tolerances of brushtail possums. She had six coppery brushtails from the Atherton Tablelands included in her captive study, and I thank Jane very much for providing me with DNA from these animals, and allowing me to come measure and photograph each of her coppery brushtails.

I discovered during the course of this research that there is a fourth colour morph of brushtail possum; the golden brushtail possum. With fur colour similar to that of a yellow labrador dog, these striking brushtails appear to have a recessive fur colour mutation. Though my attempts thus far to amplify the MC1R colour gene in these animals have been unsuccessful (ironically it seems this is due to some unlucky positioning of the very mutations I had hoped to find!), I would like to thank Wildworld nature park in Sydney for sending me DNA from one of their golden brushtails, Martin Fingland from Geckoes Wildlife, and Michael Pyne from Currumbin Sanctuary for their assistance tracking down golden brushtails and offering to provide brushtail DNA samples also.

I am grateful also for the considerable financial support extended to this project and to myself. I mentioned already the contribution by the Australia and Pacific Science Foundation, and I thank them for their investment. Thankyou also to James Cook University, for providing financial support through IRA and

Graduate Research Scheme funding, which assisted with genetic analysis in particular. I was extremely fortunate to have a living allowance in the form of an Australian Postgraduate Award stipend, and I thank the Australian Government very much for that support, as it allowed me to focus more on this study, while keeping me housed and fed.

I could not have undertaken this research without the help of volunteers. I am extremely grateful to all those who came out in the middle of the night, and helped chase possums around the rainforest on a mountainside that was an hour and a half from Cairns. Steve Ryan and Romina Rader assisted Sam Price-Rees and Katie Jones in 2005. Anthony Mann was cycling around Australia when he kindly took a detour to chase possums for a week or so. Westen Thomas and Luke Fania cheerfully endured 353 cold, wet trap nights, in which we caught only a cat, a rat and one possum. Peter Byrnes assisted me on several trips, and in turn introduced me to the Daintree Rainforest and my first musky rat kangaroos, cassowaries and a ridiculous number of dragons. Mel Commerford was amazingly cheerful and supportive on my most atrocious night darting; I consistently missed each possum that night by just an inch! Carissa Fairweather, Ilona and Pieter Moerman, Sarah Schapel and William Hancock, Denise and Gareth, Emma King, Louise Halritchie, Mandy Soymonoff, Martine Adriaansen, Madeline Ford, Laura Grogan and Sarah Meyer were all amazing volunteers, helping with distance sampling surveys, trapping, as well as darting brushtails. Tim Johns, a close friend for many years, was outstandingly tolerant on his holiday as planned visits to tourist attractions were replaced with helping me search for blackened traps in Herberton State Forest after a fire razed the new habitat gradient site I had just established. Andrew Picone came darting several times, including the first night I managed to dart two possums, and kindly allowed me up to his Mt. Lewis field site; a botanist, Andrew has given me a considerable appreciation for *Eucalyptus grandis* and for the very special Bunya pine. Natsumi Morita, Eri and Yuriko, speaking little English, were amazingly trusting as I led them off into unfamiliar bush in the middle of the night, and then, having darted a very large male brushtail that went running half-doped off into the rainforest, coped amazingly well as I yelled at them to stay on the track before dashing off into the dark after him! About five minutes later I

returned to find my three new friends looking somewhat uncertain about their situation, for which I do apologize, though I suspect it helped considerably that I did in the end return with the possum! Cherie Dugal interned with our lab and was a brilliant volunteer, assisting particularly with establishing a site near the very rainy town of Millaa Millaa.

Millaa Millaa was a fantastic place for fieldwork despite the continuous heavy rain, which unluckily, seemed to coincide with our arrival each trip! Russel Edwards, a town resident and QPWS ranger, was instrumental in establishing the Millaa Millaa site. I met Russel while tree-climbing in Ravenshoe for another of our lab's possum projects, and amusingly, it is at this same tree on numerous trips since that first meeting that we usually run into each other! Before my first trip to Millaa Millaa, Russel had talked to friends around town and gained cautious permission for me to trap around different properties. In the course of a single afternoon of introductions and setting up traps, the Millaa Millaa site was near-established. Thank you Russell for all your help, introductions and for sharing your enthusiasm about possums and tree-climbers. Thanks also to Beau and Amy, Emma and Rick, Mel, Les and Mickey who trusted me to wander around their properties at unusual hours without waking anyone up, and to trap possums without catching family pets!

Also in Millaa Millaa, I am greatly indebted to Brigitte and Georgina Humphries, who allowed me to trap around their property, helped clear the traps twice a day, and shared a passion for animals that was truly inspiring, and I thank them for sharing their time, advice and enthusiasm. Brigitte was also instrumental in setting up a visit to Millaa Millaa primary school, and I thank her and Mr. Stephen Fresta for organizing this visit. I was amazed by the enthusiasm of the kids around town, wanting to see what we'd caught each morning. At most of the properties, we'd arrive around dawn and there would be a group of children ready to see what was in the traps, even if we'd not captured anything all week! The visit to Millaa Millaa's primary school was an important opportunity to reciprocate this interest, and I thank the town of Millaa Millaa for its support, especially the children who were so taken with tales of thorny devils, giant Indonesian geckoes and little planigales.

I'd like also to thank Michael Joyce and Pat Nagellega from QPWS, who were instrumental in helping me to access Herberton State Forest after cyclone Larry hit. With so many tracks closed and faced with the formidable cleanup of these forests, Michael and Pat were incredibly supportive, and helped me greatly to access my principle field site after this destruction.

To my family and friends, who have put up with my constant talk of possums, Henry in particular, thank you so much for all your support, and for encouraging so much laughter and fun throughout my PhD project! I have been incredibly lucky to have made so many great friends at JCU in Cairns, such that it can often be difficult to feel like I'm going to work; a notion that to my mind suggests far less cheerful days. Instead, even through the longest and most tiring of days, these have been days of adventuring in the rainforest with friends, or working alongside them to solve my brushtail puzzle. And so I would like to thank Johan Larson and Gabriel Porolak, my funny, kind and enthusiastic teammates on so many rainforest adventures, and back in the lab, both of whom have scaled trees and enthusiastically traipsed through rainforests in the cold 3am darkness for my copperies! Thank you also to other lab members past and present: from Cairns, Katie Jones, for her help and encouragement as I learned to shoot, Gabriella Eiris, for her support and advice particularly in troubled PhD times, Megan Quenzer and Jeff Silverman, for so much fun in the field, and for introducing me further to the herpetological world, Toby Ross and Sue Tallarico for their encouragement and sharing amazing stories of PNG, Mel Commerford, for sharing in so much laughter and most importantly, for understanding how a few lines on a electrophoresis gel can mean celebratory dancing around the genetics lab! And in Townsville, thankyou to Stephen Kolomyjec, who taught me how to catch platypus and generously let me help weigh and measure one, Veronica Menz and Brooke Bateman, for their hospitality and warm welcome each time I came down south, sharing their mammal trapping tips, help acquiring genetics supplies, and occasional genetics commiserations.

Kylie Brown and Jenni Paul have been and continue to be the most amazing friends, and I am immensely grateful for all their advice, support and encouragement throughout this project, and for introducing lots of fun, hugs, laughter and mischief to my time here in Cairns.

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And finally to my family. It has been a long road from Melbourne to where we all are now, and throughout family triumphs and difficulties, the births and deaths over the course of doing my PhD, I have had the incredible support of my family. I thank most deeply my parents, Mandy and Stan Kerr for all their love, encouragement and support. Though I am still dubious about the complex ladder-pulley-trap system they tried to convince me would solve the problems I had early on in capturing the coppery brushtails, Mum's idea about using a net did prove very useful on several occasions; most notably as a distraction in the successful, yet highly ridiculous capture of one particular possum. I am yet to figure out how I managed in the rainforest to outrun and catch the little fellow, as it turned out that he wasn't (as I had believed at the time) under the effects of any anesthetic at all!

My amazing sister Takara has been an incredible strength, and I thank her for all her love and support, and for providing important perspective to my life. To this end I would like to thank also her husband, Shannon, and her five boys (three of whom came into the world during my time as a PhD student), Justin,

Broden, Christian, Lincoln, Bailey and Romeo for all their mischief and love and for not getting too upset at their auntie when I have had to put the possums first for a while.

Lastly, I am forever indebted to Connie Kerr, my ‘Grandma-in-Sydney’, for sharing so much love, encouragement and wisdom. In particular, for teaching me that math and music are thoroughly entwined.

ABSTRACT

I investigated the role of habitat in shaping mammalian evolution by studying the divergence of two parapatric subspecies, the common brushtail possum, *Trichosurus vulpecula vulpecula*, and the coppery brushtail possum, *T.v.johnsonii*, which are found in close proximity on the Atherton Tablelands in North Queensland, Australia. Their reputed distribution and colour differences, then unquantified, suggested that these subspecies may be candidates for evolution through parapatric speciation. This has never before been demonstrated in a mammal.

I discovered that along a habitat gradient from dry sclerophyll forest and mosaic ecotone to rainforest, brushtail possums differed significantly in fur colour. The two morphs were characterised by their fur colour saturation: its colour intensity. Possums with low colour saturation were grey and did not inhabit rainforest. Those with high fur colour saturation were a red-copper colour and did not occur in dry sclerophyll forest. There was a dichotomy in the shade of red expressed among coppery brushtails, which was either a red-orange or red-purple hue, with the different rainforest localities of brushtails significantly associated with this variation. Brushtails in ecotone were either coppery or grey. Fur colour did not vary with distance from the ecotone, nor did fur colour appear to change once established in early development. Ecotone habitats supported very few possums, which may suggest some potential for the ecotone to restrict gene flow along the habitat gradient. The population density in rainforest was 18.6 times that in ecotone, and dry sclerophyll forest supported 8.7 times more brushtails than ecotone habitat.

Coppery and grey brushtail possums were also morphologically distinct in body size. On average, coppery brushtails had ears that were 8mm shorter and 3.4mm thinner, legs that were 3.6mm shorter from knee to heel, and tails 34mm longer than grey brushtail possums of the Atherton Tablelands. There was no sexual dimorphism among brushtail possums along a habitat gradient for body size or colour, suggesting that sexual selection is unlikely to be acting upon these traits to promote divergence.

Comparing mitochondrial DNA control region sequences I found that the morphological distribution was not the result of secondary contact between reciprocally monophyletic populations: coppery and grey possums have evolved together in multiple, distantly related clades. Analyses of the morphology of these clades demonstrated that variation in body-shape morphology was not associated with genetic similarity but with fur colour. As such, possums with the same fur colour also shared body size morphology, whether they were from genetically distant clades or if they were genetically similar. These differences, together with the bimodal distribution of morphs along habitat gradients, indirectly suggested that selection is acting upon these morphological traits to produce the phenotypes and distribution observed.

Examination of the population structure of Atherton Tablelands brushtail possums using mitochondrial DNA control region sequences demonstrated that grouping populations by colour morphology did not explain genetic variation. Genetic differences between populations were not explained by the latitudinal, longitudinal, straight-line or elevation distances between them. However 30.1% of variation could be explained through the identification and separate grouping of the four populations that were closest to rainforest habitat. Along a habitat gradient, grey and coppery populations were distinct.

Investigation of population structure with microsatellite loci showed significant gene flow throughout the Tablelands. Along the habitat gradient, adjacent coppery and grey populations were genetically distinct despite this widespread gene flow. Indeed several geographically more distant populations were not distinct with these markers. This suggested that gene flow is restricted along the habitat gradient. However reproductive isolation was not complete: calculations revealed that gene are exchanged in both directions along the gradient.

Both grey and coppery brushtail possums reproduced synchronously, suggesting that there was no temporal discontinuity to gene flow. However this reproductive synchronicity may limit the potential for polygyny.

With selection acting on morphology and gene flow restricted along a habitat gradient, two possums separated by the same geographic distance should be less related if they are different morphs than if they are the same colour. However, while the pairwise relatedness between different brushtail colour morphs was significantly different to those among coppery brushtails, there was no difference to comparisons among greys. This may be a consequence of the higher population densities of coppery brushtails; relatives may possess smaller home ranges and be closer. However these genetic results did not match our theoretical expectations: possums of different colour morphs and habitats were not less related than possums of the same morph at the same distance of separation. My underlying assumption was that fur colour in the coppery brushtail possum, like so many other species, is a genetically determined trait. If true, then brushtail colour is inherited and determined via an unprecedented mechanism. While this can not be discounted, I questioned my underlying assumption.

Fur colour can also be phenotypically plastic. Climate and diet can determine fur colour, though usually only temporarily. Fur colour did not appear to change once established in early development. The presence and fine-scale distribution of coppery and grey brushtails in rainforest fragments suggested that climatic effects are highly unlikely to be determining fur colour. There has been one previous demonstration of diet permanently determining fur colour via maternal diet acting *in utero* in laboratory mice. I found that determination of brushtail fur colour by maternal diet and selection upon this trait better explains the observed morphological distribution and genetic structure. Further experimental research is needed to conclusively demonstrate this effect, to explore how widespread this capacity for coppery brushtail colour is, and to discover which foods are involved in fur colour expression. Such an congenital change would be highly significant in evolutionary research as it allows, without mutation, the widespread single generation adaptation of offspring to the environmental conditions experienced by the parent.

TABLE OF CONTENTS

LIST OF FIGURES