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Divergence of a mammal along a habitat gradient:
A study of the coppery brushtail possum, *Trichosurus vulpecula johnsonii*.

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in December 2011

For the degree of Doctor of Philosophy
in the School of Marine & Tropical Biology
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STATEMENT OF ACCESS

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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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Sarah Emily Kerr
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 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
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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.

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I was very fortunate to receive guidance from Associate Professor Steve
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Jane DeGabriel was conducting research at James Cook University’s
Townsville campus, studying the dietary tolerances of brushtail possums. She
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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
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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 Halritchlie, Mandy Soymonoff, Martine Adriaanssen, 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!

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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.
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