

# JCU ePrints

This file is part of the following reference:

**Koetz, Anna Helene (2007) *The causes & evolutionary consequences of behavioural, morphological & molecular genetic variation in the Chowchilla, *Orthonyx spaldingii**. PhD thesis, James Cook University.**

Access to this file is available from:

<http://eprints.jcu.edu.au/3218>

**The causes & evolutionary consequences of  
behavioural, morphological & molecular genetic variation  
in the Chowchilla, *Orthonyx spaldingii*.**

Thesis submitted by  
Anna H el ene Koetz B.Sc. (Hons) JCU Qld  
in May 2007

For the degree of Doctor of Philosophy  
in the School of Marine & Tropical Biology  
James Cook University

## Statement of Access

---

I, the undersigned, author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and I do not wish to place any further restrictions on access to this work.

.....

(Signature)

(Date)

## Declaration

---

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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

.....

(Signature)

(Date)

## Electronic copy

---

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library is an accurate copy of the print thesis submitted, within the limits of the technology available.

.....

(Signature)

(Date)

## 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 number A870\_04).

.....

(Signature) (Date)

## Acknowledgements

---

I could not have come this far without the generous help of my parents, Imi & Didi Kötz, who are by far the most skilled Chowchilla-catchers out there. Their enthusiasm, patience and persistence in getting those birds into the net are unrivalled, even by me. Thank you so much for your love and support, and endless hours of recording, catching and putting up with my tantrums. You are truly special parents!

I also cannot thank enough my other half and best friend, Lachy Trowse, who also had to put up with the unbelievable and unpredictable highs and lows of fieldwork in particular, and of doing a PhD in general. I could not have come this far without his patience, love and support. Thank you so much!

I would like to send a huge thank you to my dear friend Sandra Abell. She has been there for me from the start (and before), in the field as well as at uni, and I am eternally grateful for her support and advice, pretty much every single day since I started. I am so glad to have you as a friend! I would also like to thank Kylie Anderson for her advice, support and great enthusiasm both in the field and at uni. Thank you so much, Sandra and Kylie!

I also wish to sincerely thank Darren Peck for his advice and wisdom about everything genetic, and Pramana Yuda for many genetic discussions. Thanks to Darren and Pram I made it through the genetic jungle reasonably unharmed. Thank you also to everyone in the molecular genetics lab, particularly Lynne Jones, for generous help and advice.

I also had the help of many enthusiastic people who assisted in recording and catching Chowchillas. Thank you Greta Kading, Lauren Cartledge, Liz Poon, Rupert Russell, Carol Erwin, Avril Underwood, Ian Northcott, Madeleine Ford, Donna Baldwin, Kris Corvino, Kate Webster, Philip Newey, Damian Riggs, Gareth Owens, Ben Jones and Jacqui Acourt.

I also wish to thank several experts from overseas for extremely interesting and insightful discussions about bird song: Hans Slabbekoorn, Jacintha Eilers, Robert Lachlan and Peter McGregor.

Last but definitely not least, I wish to give a very big thank you to my supervisors Brad Congdon and David Westcott. Thank you, David, for introducing me to the fascinating world of bird song, and for all your help and advice along the way. And Brad, an extra special thank you for your patience, focus and enthusiasm, and for your invaluable guidance, advice and wisdom throughout my degree!

❧❧❧

" A first encounter with Chowchillas may occur without a single bird in view, just a few yodelling notes being enough to summon attention. Then, with luck, a Chowchilla will give a full performance in fine resonant tones: whooping, gobbling, yodelling – a rush of sound fit almost to shake leaves from the trees. The effect is hugely cheering and invigorating, the more so when other birds in the group add to the performance, inspired by the bird which initiated the singing. A listener can then move quietly forward and may be able to see a bird in full voice as it stands well up, chest and throat pumping strongly to fling the notes out, head jerking back and forth a little in emphasis . . . . "

❧❧❧

An excerpt from "Amongst Trees – Images from the Rainforests of North-east Queensland" (Russell *et al.* 2006)



**Plate 1. Female Chowchilla, *Orthonyx spaldingii melasmenus*, engaging in territorial song at Mt. Lewis.**



# Table of contents

---

STATEMENT OF ACCESS .....	2
DECLARATION .....	3
ELECTRONIC COPY .....	4
DECLARATION ON ETHICS.....	5
ACKNOWLEDGEMENTS.....	6
TABLE OF CONTENTS.....	8
LIST OF TABLES.....	11
LIST OF FIGURES.....	12
LIST OF PLATES .....	14
ABSTRACT .....	15
<b>CHAPTER 1 INTRODUCTION.....</b>	<b>18</b>
1.1 VARIATION AND GENETIC EVOLUTION .....	18
1.2 VARIATION AND CULTURAL EVOLUTION .....	18
1.3 BIRD SONG AS A CULTURAL TRAIT .....	19
1.3.1 Natural selection.....	20
1.3.2 Sexual selection.....	21
1.3.3 Genetic & cultural drift .....	21
1.4 THE STUDY SYSTEM.....	22
1.5 AIMS AND THESIS STRUCTURE .....	24
<b>CHAPTER 2 LARGE-SCALE SONG DIVERGENCE.....</b>	<b>26</b>
2.1 INTRODUCTION .....	26
2.2 METHODS .....	28
2.2.1 Study Sites.....	28
2.2.2 Song Recordings .....	29
2.2.3 Spectrotemporal Song Measurements.....	30
2.3 RESULTS .....	33
2.3.1 Chowchilla song.....	33
2.3.2 Spectrotemporal Song Measurements.....	36
2.4 DISCUSSION .....	40
<b>CHAPTER 3 SMALL-SCALE SONG VARIATION .....</b>	<b>44</b>
3.1 INTRODUCTION .....	44
3.2 METHODS .....	46
3.2.1 Study Sites.....	46
3.2.2 Song Recordings .....	47

3.2.3	Syllable Classification.....	47
3.2.4	Syllable Analysis.....	49
3.3	RESULTS .....	50
3.3.1	Syllable Classification.....	50
3.3.2	Syllable Sharing .....	52
3.4	DISCUSSION .....	54
<b>CHAPTER 4 MORPHOLOGICAL AND GENETIC DIVERGENCE.....</b>		<b>60</b>
4.1	INTRODUCTION .....	60
4.2	METHODS .....	62
4.2.1	Morphological analyses.....	62
4.2.2	Molecular analyses.....	63
4.2.3	Phylogenetic analyses .....	64
4.2.4	Divergence times.....	65
4.3	RESULTS .....	66
4.3.1	Spatial patterns of morphological variation .....	66
4.3.2	Spatial patterns of genetic variation .....	69
4.3.3	Divergence times.....	73
4.4	DISCUSSION .....	73
<b>CHAPTER 5 THE EVOLUTIONARY CONSEQUENCES OF SONG VARIATION .....</b>		<b>77</b>
5.1	INTRODUCTION .....	77
5.1.1	Large-scale song discrimination.....	77
5.1.2	Small-scale song discrimination.....	78
5.2	METHODS .....	80
5.2.1	Study sites & recordings .....	80
5.2.2	Playback design.....	80
5.2.3	Test stimuli.....	82
5.2.4	Response measures.....	83
5.2.5	Statistical Analyses .....	83
5.3	RESULTS .....	84
5.4	DISCUSSION .....	87
5.4.1	Small scale implications (local vs. distant dialects) .....	88
5.4.2	Medium scale implications (within-subspecies dialects) .....	89
5.4.3	Large scale implications (between sub-species dialects) .....	90
<b>CHAPTER 6 DISCUSSION .....</b>		<b>92</b>
6.1	SYNTHESIS.....	92
6.1.1	Large-scale song variation.....	92

6.1.2	Small-scale song variation .....	92
6.1.3	Molecular genetic divergence .....	93
6.1.4	Morphological divergence.....	93
6.1.5	Sexual dimorphism.....	94
6.1.6	Geographic song discrimination.....	94
6.2	CONCLUSIONS .....	94
6.3	FUTURE RESEARCH .....	95
	REFERENCES.....	96

## List of Tables

---

Table 2.1. Latitudes and longitudes of recording locations within each refuge and area of recolonisation. Number of sites within Lamb Range locations shown in brackets. ....	28
Table 2.2. Average distances (km) $\pm$ 2SE between sampling refuges, locations and sites. The number of between site-comparisons ( <i>N</i> ) from which the data was derived is given. ....	29
Table 2.3. Average spectrotemporal measurements $\pm$ SD for 773 Chowchilla songs recorded in five refugia (TU, CU, LR, WH, CR) and two areas of post-glacial recolonisation (BMC, PR) in the Wet Tropics, Australia. Spectrotemporal measurements include duration (secs), peak frequency, minimum frequency and bandwidth (Hz). ....	34
Table 2.4. Correlation (loading) matrix of all song variables used in the NMS analysis; <i>r</i> , <i>r</i> <sup>2</sup> and <i>tau</i> given for Dimension 1 and 2. <i>R</i> <sup>2</sup> of variables influencing song variation the most are indicated in bold. ....	36
Table 3.1. Results of the Principal Components Analysis using 19 acoustic characteristics of each syllable in the syllable classification. Components with Eigenvalues >1 are shown, including the percentage and cumulative percentage, main influencing factors and general type of those factors. Components were rotated using Varimax Rotation ....	52
Table 4.1. Patterns of morphological variation in Chowchillas north and south of the Black Mountain Corridor (N/S) and between the sexes; PC1 is a composite body size measure derived from a Principal Components Analysis. <i>F</i> and <i>p</i> are given for the two factors (N/S and Sex) as well as for the interaction effect. ....	67
Table 4.2. Variable nucleotide sites for the 14 haplotypes found in mtDNA control region sequences of 62 Chowchillas; number of individuals found with each haplotype are given for each geographical area. N – Northern Uplands (TU, CU & BT), AU – Atherton Uplands (LR & AU), CR – Cardwell Ranges (CR & WH), and PR – Paluma Ranges.....	69
Table 4.3. Pair-wise <i>F</i> <sub>ST</sub> comparison matrix for four Chowchilla populations: North, Atherton Uplands (AU), Cardwell Ranges (CR) and Paluma Ranges (PR). Significant comparisons (AMOVA: <i>p</i> < 0.0001) are indicated by an asterisk. ....	71
Table 4.4. Neutrality, diversity and expansion indices including expansion time estimates for Chowchilla mtDNA control region sequences. Significant values are indicated in bold (* <i>p</i> < 0.05, ** <i>p</i> < 0.0001). Patterns expected under selection or expansion are also given.....	72
Table 5.1. Average response strength ( $\pm$ SD), measured as inverse approach latency, inverse song latency and closest approach distance (m) for each dialect stimulus. Test dialects ‘local’, ‘other site’, ‘other location’ and ‘other refuge S’ are all from within the same subspecies ( <i>O. sp. spaldingii</i> ); test dialect ‘other refuge CU’ and ‘other refuge TU’ are from the different subspecies ( <i>O. s. melasmenus</i> ).....	86

## List of Figures

---

- Figure 1.1. Map showing a) Pleistocene refugia as proposed by Webb & Tracey (1981; black shading), and northern/southern isolates as proposed by Nix & Switzer (1991; grey shading); and b) locations of sampling sites (white circles) across the current Wet Tropics rainforests (grey shading), in north-eastern Australia. BT – Big Tablelands, TU – Thornton Uplands, WU – Windsor Uplands, CU – Carbine Uplands, BMC – Black Mountain Corridor, LR – Lamb Range, AU – Atherton Uplands, WH – Walter Hill Ranges (w – west & e – east), CR – Cardwell Range, SR – Seaview Range, PR – Paluma Range; BMC and PR represent areas of post-Pleistocene recolonisation.....24
- Figure 2.1. Song spectrogram of a Chowchilla song from the Lamb Range (Davies Creek dialect), showing the song’s waveform (upper figure) and power spectrum (left figure); each black trace on the spectrogram itself denotes one element, which was counted to determine the number of elements and the element rate of that song; the frequency parameters used for further analysis were measured at the mean spectrum of the whole song using Avisoft SASLab Pro (Specht 2005) and are highlighted within the spectrogram: (a) start of song, (b) end of song, (c) peak frequency (frequency with the most energy as shown on power spectrum, Hz), (d) minimum frequency (Hz), (e) bandwidth (difference of maximum and minimum frequency, Hz).....31
- Figure 2.2. Example spectrograms of seven Chowchilla songs from five different refugia and two areas of recolonisation (a – TU; b – CU; c – BMC; d – LR; e – WH; f – CR; g – PR) within the Wet Tropics of Australia. Position of the Black Mountain Corridor (BMC) indicated by dashed line. ....35
- Figure 2.3. Ordination plot of the two significant NMS dimensions (Dimension 1 –Bandwidth; Dimension 2 – Peak frequency) using six spectrotemporal song variables of Chowchilla song; songs from each refuge and area of recolonisation\* are represented by their centroid  $\pm 2$  S.E. (TU – Thornton Uplands, CU – Carbine Uplands, BMC\* – Black Mountain Corridor, LR – Lamb Range, WH – Walter Hill Ranges, CR – Cardwell Range, PR\* – Paluma Range). .....37
- Figure 2.4. Ordination plot of the two significant NMS dimensions (Dimension 1 –Bandwidth; Dimension 2 – Peak frequency) using six song variables of Chowchilla song; songs from each refuge and area of recolonisation\* are represented by their centroid  $\pm 2$  S.E. (TU – Thornton Uplands, CU – Carbine Uplands, BMC\* - Black Mountain Corridor, LR – Lamb Range, WH – Walter Hill Ranges, CR – Cardwell Range, PR\* – Paluma Range). ....38
- Figure 2.5. Ordination plot of the two significant NMS dimensions (Dimension 1 –Bandwidth; Dimension 2 – Peak frequency) of Chowchilla song; songs from each location are represented by their centroid  $\pm 2$  S.E. and identified by their respective vegetation type following Tracey’s (1982) vegetation classification (Vegetation type 1 a & b – open circle; Type 2 a – filled circle; Type 8 – grey circle). ....39
- Figure 2.6. Pair-wise comparison of song dissimilarity (squared Euclidean distances) vs. geographic distance (km) for between/within refuge comparisons (within refuge comparison – closed circles, between refuge comparison – open circles); line of best fit for between-refuge comparisons (Mantel  $r = 0.393$ ,  $p = 0.011$ ). ....39
- Figure 2.7. Linear regression of Chowchilla tarsus length (a) and bill length (b) vs. peak frequency (filled circles) and bandwidth (open circles); data for birds from north of the BMC are indicated in grey (peak frequency – filled circles, bandwidth – open circles); all relationships were non-significant (tarsus length vs. peak frequency and bandwidth:  $r^2 = 0.272$ ,  $F = 2.241$ ,  $p = 0.185$ , and  $r^2 = 0.007$ ,  $F = 0.046$ ,  $p = 0.837$ , respectively;  $\log$  bill length vs. peak frequency and bandwidth:  $r^2 = 0.023$ ,  $F = 0.139$ ,  $p = 0.723$ , and  $r^2 = 0.018$ ,  $F = 0.111$ ,  $p = 0.750$ , respectively).....40
- Figure 3.1. Three models of the relationship between song similarity and distance between individuals (modified from Wilson *et al.* 2000); this distance may be number of territories or geographic

distance (e.g. meters); model A is indicative of post-dispersal and/or open ended learning, model B is indicative of pre-dispersal and/or closed-ended learning, and model C is indicative of non-random dispersal or active avoidance (Wilson <i>et al.</i> 2000). .....	46
Figure 3.2. Examples of Chowchilla dialects from the Thornton Uplands (a), the Black Mountain (b), the Lamb Range (c-g) and Paluma Range (h). Spectrograms show songs from one of each site at a) Thornton Upland, b) Black Mountain, c) Douglas Track, d) Clohesy Fig, e) Clohesy River Road, f) Lake Morris, g) Davies Creek, g) Kauri Creek Road and h) Paluma dam. ....	48
Figure 3.3. Chowchilla syllable examples, taken from the syllable/element library; syllables were classified into 5 broad categories, A, B, C, D and W; within those categories, syllables were grouped by similarity of frequency and shape. Numbers underneath syllables are syllable identifiers. ....	51
Figure 3.4. Song sharing index vs. geographic distance (km) for Chowchilla song; a) 0 – 30 kilometres; b) within site (= neighbours; closed squares) and between site (= non-neighbours; open squares) comparisons of up to 15 territories or 3 kilometres. Outliers are marked by dashed line (refer to Discussion for details). ....	53
Figure 3.5. Song sharing index ( $\pm 2$ SE) for immediate neighbours, other neighbours and non-neighbours (different site & different location) for within and between-site comparisons of Chowchilla song in the Lamb Range. Different letters indicate a significant difference, whereas the same letters indicate a non-significant difference at $\alpha = 0.05$ . ....	54
Figure 4.1. Morphological variation in male (■) and female (□) Chowchillas north (N) and south (S) of the Black Mountain Corridor; a) mean composite body score (PC1 score) $\pm 2$ SE; b) mean bill length $\pm 2$ SE. ....	68
Figure 4.2. a) Map of the Wet Tropics World Heritage Area ('Wet Tropics') showing the current distribution of rainforest habitat (light grey) and sampling sites (northern refugia 1-3, BMC 4, southern refugia a-f). b) Parsimony network for mtDNA control region sequences of Chowchillas generated by nearest-neighbour joining; circle size denotes relative haplotype frequencies, circle colour denotes location north (black) or south (white) of the BMC, and dashed lines indicate potential homoplasies. 1 – FU, 2 – TU, 3 – CU, 4 – BMC, a – LR, b – AU, c – Mt. Baldy, AU, d – WH, e – CR, f – PR (refer to text for abbreviations).....	70
Figure 4.3. Observed and expected mismatch distribution for Chowchilla mtDNA control region sequences under the expectations of the sudden expansion model (Avise <i>et al.</i> 1987): a) populations north of the BMC, and b) pooled populations south of the BMC. ....	72
Figure 5.1. Map of the current distribution of the Wet Tropics World Heritage rainforest. Dark grey indicates the distribution of <i>Orthonyx spaldingii melasmenus</i> north of the BMC, lighter grey indicates the distribution of <i>O. s. spaldingii</i> south of the BMC. Locations of test-dialects across the Chowchilla's range are indicated by white circles. Insert depicts a map of the Lamb Range and the position of the three playback locations (CF, DC & KC).....	81
Figure 5.2. Percentage of trials in which Chowchillas responded by approaching (white bars) and singing (grey bars) to different dialect stimuli. Light grey bars indicate test dialects from within the same subspecies, whereas dark grey bars indicate test dialects from the different subspecies (north of the Black Mountain Corridor). Numbers in the bars denote sample sizes. ....	85
Figure 5.3. Inverse time to response by song (song latency $\pm 2$ S.E.) and similarity of the song dialect to the test groups' local song (song similarity – grey bars). Light grey bars indicate similarity of test dialects from within the same subspecies, whereas dark grey bars indicate similarity of test dialects between subspecies. ....	86
Figure 5.4. Relative response of the sexes to different dialects. Bars indicate the percent of males (light grey) or females (dark grey) responding by approaching first (a) or closest (b); sample sizes for each dialect are indicated. ....	87

## List of Plates

---

Plate 1. Female Chowchilla, *Orthonyx spaldingii melasmenus*, engaging in territorial song at Mt. Lewis.  
.....7

## Abstract

---

Intra-specific variation provides the basis on which evolutionary processes such as genetic drift, natural and sexual selection can act, creating distinct patterns of divergence within and among populations. Intra-specific variation and population divergence form the very beginning of the speciation process, and so determining the relative influence of different evolutionary processes in influencing current patterns of divergence is crucial in clarifying the mechanisms of the speciation process. Although most research focuses on patterns of genetic and morphological divergence, populations may also show divergence in cultural (learnt) behaviours such as song. Divergence in song is particularly intriguing, because it is crucially important in species and mate recognition as well as in sexual selection. In true songbirds (Oscines), songs are acquired from conspecifics through learning and imitation, defining bird song as a cultural trait that is non-genetically transmitted. Because song is learned culturally, inaccurate copying of the tutor song is possible and some level of spatial variation in song is inevitable. This, in turn, can lead to cultural evolution. Cultural and genetic evolution have many parallels and similarities, including the processes of mutation, drift, natural and sexual selection and cultural flow. Geographic variation in song could potentially influence population genetic divergence leading to speciation, by favouring within-dialect mating and natal philopatry, and discouraging between-dialect dispersal. Nevertheless, the notion that divergence in a cultural trait may promote speciation is still highly controversial.

Thus, the goals of this project were to determine the relative importance of different evolutionary forces in promoting geographic variation in song of the Chowchilla, *Orthonyx spaldingii* (Corvoidea), and to clarify the function and possible evolutionary consequences of such variation. In order to distinguish between the influence of different evolutionary forces, an integrative approach was used that combined patterns of genetic, and morphological variation with patterns of song variation. Therefore, the aims of my PhD were to (1) quantify the extent and pattern of large-scale geographic variation in song, morphology and neutral molecular genetic markers across the Chowchilla's entire range; (2) determine the evolutionary, cultural and social processes influencing both large and small-scale variation in song in order to clarify the functional significance of song variation; and (3) determine whether birds discriminate between local, distant and foreign song dialects to further establish the possible functions as well as evolutionary consequences (i.e. reduced gene flow or reproductive isolation) of geographic variation in song.

The Chowchilla is a rainforest specialist bird endemic to the montane tropical rainforests (the "Wet Tropics") of north-eastern Australia. The Chowchilla is remarkable for its striking, yet previously unstudied large-scale geographic variation in song, which is sung by both males and females alike in



territorial encounters. The Wet Tropics rainforests of Northern Australia experienced range contractions and expansions during the Pleistocene climatic fluctuations. As a consequence, many wet tropics endemic taxa are genetically divergent across an old climatic barrier, the Black Mountain Corridor (BMC), which has intermittently separated the northern and southern Wet Tropics. Limited evidence of morphological and molecular genetic divergence across the BMC also exists for the Chowchilla. Evidence of diversification in several character traits (molecular genetic, morphology and song) combined with the well established pattern of habitat expansion and contraction in the wet tropics, means that the Chowchilla provides an ideal and unique model system for comparing the evolutionary forces driving song variation with those thought to influence genetic and morphological divergence.

I recorded Chowchilla songs from 15 locations across the species' range, covering five historically isolated populations (Pleistocene refugia) and two areas of post-Pleistocene recolonisation. I measured six spectral characteristics of 773 songs and used a multivariate approach to test for large-scale song divergence within and among refugia, as well as across the BMC. Songs were also divided into their syllables, and a syllable catalogue was created for the whole population. Pair-wise comparisons of syllable sharing were then used to analyse song similarity at smaller spatial scales. I also collected blood samples and morphological measurements from 54 Chowchillas captured across their range. Morphological measurements were analysed using a Principal Components Analysis to determine the extent of morphological divergence among populations from different refugia, across the BMC, as well as between the sexes. Mitochondrial DNA (mtDNA) control region sequences from captured birds and museum specimens were analysed to determine the population genetic structure. Finally, I conducted playback experiments to determine whether Chowchillas differentiate between song variants from increasingly distant and isolated populations.

I found that historically isolated populations could be clearly distinguished by their spectrotemporal song characteristics, particularly bandwidth and peak frequency. I also found striking song divergence across the BMC. Northern refugia showed significantly narrower bandwidths and higher peak frequencies than southern refugia. Song characteristics were not influenced by geographic distance, habitat type or body size. Thus, given the known history of population isolation, song characteristics were most likely influenced by vicariant isolation in refugia followed by cultural drift. Chowchillas also showed exceptional small-scale variation in song syllable characteristics. Within historic refugia, song similarity was significantly correlated with distance. It was highest amongst neighbours and decreased sharply at one kilometre. These results are consistent with the idea of post-dispersal song learning, and reveal a strongly territorial function of song dialects.

Patterns of molecular genetic and morphological differentiation mirrored the pattern of large-scale song divergence across the Chowchilla's range. This suggests that historical isolation during

Pleistocene glaciation divided the Chowchilla into two distinct molecular lineages (northern and southern) that also have clearly divergent morphology and song characteristics. These findings demonstrate that vicariant isolation and genetic drift are sufficient to produce molecular genetic and phenotypic divergence in a rainforest specialist taxa. I also found significant and consistent sexual dimorphism in size and plumage colour in both northern and southern lineages, despite major size differences between lineages. This suggests that sexual selection as well as genetic drift have been important in shaping current patterns of morphological variation in both lineages. I also found no equivalent variation in bill morphology across the species entire range, suggesting that natural, stabilising selection associated with a specialized feeding niche may maintain bill characteristics in this species, independent of other morphological change.

Finally, playback experiments showed that Chowchillas recognised and approached their species-specific song in most playback trials irrespective of its origin. However, Chowchillas discriminated between local and foreign songs. Within lineages, groups sang significantly sooner and more often when hearing a local dialect *vs.* more distant song dialects. These results also support the territorial function of small-scale song variation. However, song of the alternative lineage elicited an unexpectedly high number of territorial responses. As it is highly likely that this territorial song also serves as a mate choice and advertisement function, recognition of foreign Chowchilla song means that song is unlikely to serve as a pre-mating barrier to gene flow in this species.

In conclusion, these results clearly show that all three evolutionary processes – genetic drift, natural and sexual selection – have concurrently influenced Chowchilla populations, creating contemporary patterns of divergence and variation in song, molecular genetic and morphological character traits, particularly across an old climatic barrier pre-dating the Pleistocene. Nevertheless, despite clear divergence in these traits, northern and southern populations have not diverged sufficiently to create a behavioural, pre-mating barrier to gene flow.