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

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

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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).

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" 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.

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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 though 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.