

Skull shape of a widely-distributed, endangered marsupial reveals little evidence of local adaptation between fragmented populations

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Abstract

The biogeographical distribution of diversity among populations of threatened mammalian species is generally investigated through population genetics. However, intraspecific phenotypic diversity is rarely assessed beyond taxonomy-focused linear measurements or qualitative descriptions. Here, we use a technique widely used in the evolutionary sciences – geometric morphometrics – to characterize shape diversity in the skull of an endangered marsupial, the northern quoll, across its 5,000 km distribution range along the northern Australian coast. Skull shape is a proxy of feeding, behaviour, and phenotypic differentiation, allowing us to ask if populations can be distinguished and if patterns of variation indicate adaptability to changing environmental conditions. We analysed skull shape in 101 individuals across the four mainland populations and several islands. We assessed the contribution of population, size, sex, rainfall, temperature, and latitude/longitude to skull shape variation through Principal Components, Procrustes ANOVA, and variation partitioning analyses. Regardless of land area inhabited, northern quoll populations harbour similar amounts of broadly overlapping skull shape variation. Size predicted skull shape best, coinciding with braincase size variation and differences in the cheekbone shape. Size-adjusted population differences explained less variation with far smaller effect sizes, relating to changes in insertion areas of masticatory muscles, as well as the upper muzzle and incisor region. Climatic and geographic variables contributed little or nothing. Strikingly, the vast majority of shape variation - 76% - remained unexplained. Our results suggest a uniform within-species scope for shape variation, possibly due to phenotypic plasticity or allometric constraints. The lack of local adaptation indicates that cross-breeding between populations will not reduce local morphological skull (and probably general musculoskeletal) adaptation because none exists. However, the potential for heritable morphological variation (e.g. specialization to local diets) seems exceedingly limited. We conclude that 3D geometric morphometrics can provide a comprehensive, statistically rigorous phenomic contribution to genetics-based conservation studies.

INTRODUCTION

The conservation of mammalian diversity is an urgent issue globally (Bowyer et al., 2019; Crooks et al., 2017), but population declines have been particularly precipitous in Australian marsupials (Baker & Dickman, 2018; Fisher et al., 2014; Woinarski et al., 2015; Ziemicki et al., 2015). A high proportion of marsupials reached high levels of vulnerability in the last century, making them a particular conservation concern (Woinarski et al., 2011). Consequently, high priority conservation efforts are underway for over one hundred threatened Australian mammals (Legge et al., 2018).

One of the challenges of current conservation efforts is the determination of within-species diversity. Determining population units for management plans ensures the preservation of evolutionary potential in en-

dangered species (Crandall et al., 2000; Moritz, 1994) and the functioning of ecosystems (Des Roches et al., 2018). Population units are largely determined through molecular data (Allendorf, 2017) – e.g., endangered species of squirrels (Finnegan et al., 2008), jaguars (Wultsch et al., 2016) and wolves (Hindrikson et al., 2017) have all relied on genetics to identify their population diversity for conservation purposes. This genetic management has established links between diversity metrics and population fitness; however, it does not assess the phenotypic variation within a species and is therefore blind to organismic diversity within a population (Wanninger, 2015). This results in the potential for serious disjuncts between phenotypic intraspecific variation and genotype variability (Boyko et al., 2010; Le Rouzic & Carlborg, 2008; Vogt et al., 2008).

Understanding the phenotypic diversity among fragmented populations can provide valuable information to conservation management. In particular – in analogy to the interpretation of genetic distances – morphological differences between populations may indicate local adaptation (Colangelo et al., 2012; Meloro, 2011; Meloro et al., 2017). Current conservation studies of endangered taxa rarely use morphological data to determine phenotypic differentiation below the species level (e.g., Dierickx et al., 2015; Wilting et al., 2015); and most quantitatively rigorous assessment of phenotypic differentiation remains the domain of taxonomic studies (Celik et al., 2019; Meloro et al., 2017; Nicolosi & Loy, 2019; Senczuk et al., 2018; Sveegaard et al., 2015). Therefore, quantifying morphological variation within a species represents a largely untapped potential for understanding the phenotypic variation between taxonomic units; test hypotheses of adaptation and relatedness within a species; and provide a valuable tool for management, for example in assessing if population units may be too morphologically divergent to be cross bred in outbreeding conservation efforts. It can also inform predictions of morphological change during future species’ fragmentation events, which is a common consequence of human activity (Bennett & Saunders, 2010; Haddad et al., 2015; Hansen et al., 2013).

The anatomical complex with the most comprehensive amount of quantifiable morphological information is the mammalian skull. This is reflected in a long tradition of using linear skull measurements for taxonomic purposes (Baker et al., 2015; Cardini, 2013; Travouillon, 2016; Van Dyck, 2002). The shape of the mammalian skulls contains information on animal function (Hanken & Hall, 1993), such as masticatory loading (Herring et al., 2001), acting as a proxy for dietary behaviours in mammals (Maestri et al., 2016; Marroig & Cheverud, 2005; Nogueira et al., 2009), including marsupials (Mitchell, Sherratt, Ledogar, et al., 2018; Wroe & Milne, 2007). This is particularly relevant in the context of marsupial mammals, whose skull might not be as adaptable as that of placental mammals due to a development constraint on skull shape variation (Goswami et al., 2012; Porto et al., 2013; Sánchez-Villagra et al., 2008; Weisbecker et al., 2008, 2019). This is because marsupials are born at an extremely immature (altricial) state, but with a highly developed oral apparatus adapted to immediate and extensive feeding at the mother’s teat. This seems to reduce the potential of the oral region to diversify, both developmentally (Goswami et al., 2016) and evolutionarily (Porto et al., 2013; Sánchez-Villagra et al., 2008; Weisbecker et al., 2008). Such a developmental constraint may reduce the ability of marsupials to adapt their skull morphology at the level of within-species variation, leaving adaptation through changes in size as the only avenue of heritable adaptive shape (Marroig & Cheverud, 2005, 2010; Porto et al., 2013; Shirai & Marroig, 2010).

In this study, we use geometric morphometric analyses to provide the first population-level study of morphological population variation in the skull of a mammal of particular conservation concern. We focus on the endangered Northern quoll (*Dasyurus hallucatus* : Gould, 1842), a guinea pig-sized carnivorous marsupial with well-understood genetic differentiation between populations (Cardoso et al., 2009; Firestone et al., 2000; Hill & Ward, 2010; Hohnen et al., 2016; How et al., 2009; Woolley et al., 2015) but no information on morphological adaptation. Northern quolls appear to have had a pre-colonial distribution over 5000 km across northern Australia (Braithwaite & Griffiths, 1994). They are now separated by major biogeographic breaks into four mainland populations with no apparent gene flow (Hill and Ward, 2010) and several island populations (Woinarski et al., 1999). Northern quolls are also a suitable study system for this investigation because they inhabit a wide range of habitats, ranging from rainforests to deserts (Begg, 1981; Moore et al., 2019; Oakwood, 2002). They are opportunistic foragers of small vertebrates, invertebrates, fruit and carrion

(Dunlop et al., 2017). The species is also expected to evolve quickly because, as a semelparous species, most males die off in their first year after mating (Oakwood et al., 2001).

We capture fine-scale morphological differences of the cranium using 3D geometric morphometrics, which differs from traditional taxonomic morphometrics (Baker & Van Dyck, 2015; Travouillon et al., 2019) by being agnostic to expected shape differences and by allowing the shape variation of the whole skull to be described in high detail. This process also has the ability to provide statistical significances of shape variation patterns alongside visualisations of exactly what the shape variation in question is, thus permitting a finely resolved dissection of the drivers of shape variation that is not possible with conventional linear measurements.

We examine several potential drivers of northern quoll skull shape variation. We expect shape differences between populations to be a main part of overall skull variation. If variation relates to heritable adaptation to local environments, for example through dietary differences between high and low rainfall areas (Dunlop et al., 2017), we expect differences between populations to increase with either increased geographical distance, according to how individuals are related, or depending on local environmental differences (here accounted as rainfall and temperature). Alternatively, if local adaptation is prevented by a constraint due to the quoll's early birth, most shape variation is expected along a size gradient or should be unexplained. Lastly, it is also possible that most variation relates to the biomechanical use of the cranium in feeding and – particularly in males – biting. This would result in a mostly uniform distribution of shape variation across geographic range of northern quolls.

MATERIALS AND METHODS

Data collection (3D acquisition)

We virtually reconstructed 101 crania of adult individuals of *Dasyurus hallucatus* – including males and females from four mainland populations (Pilbara, Kimberley, Northern Territory and Queensland) and other island populations (Groote Eylandt and other small islands). Adult status was determined through incisor wear (Oakwood, 2000) and P3 eruption (Woolley et al., 2013). We 3D-scanned most of the specimens from museum collections (Queensland Museum, Australian Museum, Western Australian Museum, Australian National Wildlife Collection and American Museum of Natural History) with a GoMeasure 3D HDI109 blue light surface scanner (LMI Technologies Inc., Vancouver, Canada). Each cranium was placed in 3 different orientations on a motored rotary table that turned every 45 degrees (8 rotations per round). The 24 resulting 3D images (8 rotations x 3 orientations) were then meshed together with the scanner's software (FlexScan3D 3.3) to export a complete 3D image of each skull. This file was then treated in for hole filling (so as to not affect the biological shape of the structure), mesh decimation (to facilitate computation) and mesh reformatting (as ".ply" files need to be in binary format for subsequent importations of the mesh in R). Several photos of each specimen were also taken to help identify landmarks by discriminating biological structures from 3D artefacts in the landmarking process. Seven fully fleshed specimens from the Groote Eylandt population were CT-scanned at the Centre for Advanced Imaging at The University of Queensland in a micro CT-scanner. In order to obtain the 3D model, segmentation of the DICOM grayscale images provided by the micro CT-scan was performed with Mimics Research version 20.0. All 3D models can be accessed through MorphoSource. The University of Queensland animal ethics committee (permit number SBS/009/16/ARC) and the Northern Territory Parks and Wildlife Commission (permit number 58566) approved the research methods and the collection of the Groote Eylandt specimens.

Template creation

The template consists of 900 landmarks: 101 fixed landmarks, 93 curves (271 semilandmarks), and 18 surfaces (528 semilandmarks) (Supplementary Figure 1). For the anatomical definitions of landmarks, please refer to Supplementary Table 1. The number of curve or surface semilandmarks was determined by the complexity of the curves or area covered. High density landmark and semilandmark configurations, ranging from 800 to more than 1000 landmarks, have been demonstrated empirically to successfully capture genuine biological signal (Cornette et al., 2013; Dumont et al., 2016; Goswami et al., 2019; Gunz & Mitteroecker, 2013; Segall et al., 2016; Watanabe et al., 2019; Weisbecker et al., 2019)

To ensure the repeatability of landmarking of the manually placed fixed landmarks, three morphologically close specimens were digitised at ten repetitions. Inter-repetition variation was much lower than inter-individual variation, confirming the good repeatability of the template used in this study (Supplementary Figure 2).

Landmarking and sliding

Each 3D model was landmarked in Viewbox version 4.0 (dHAL software, Kifissia, Greece; <http://www.dhal.com>; Polychronis et al., 2013). One operator (first author) manually placed the fixed landmarks and curves. Curve semilandmarks were placed equidistantly and then were allowed to slide along their respective curves. Surface semilandmarks were placed following a thin-plate spline interpolation between the template and each specimen, followed by a projection to the surface of the 3D-model and the sliding procedure. Sliding was performed by minimizing the bending energy.

Analysis

Raw coordinate data were analysed in R version 3.6.1 (R Core Team, 2019) with the ‘geomorph’ (version 3.1.2) (Adams & Otárola-Castillo, 2013) and the ‘Morpho’ (version 2.7) (Schlager et al., 2019) packages. A Generalized Procrustes Analysis (GPA) was performed on the raw landmarks to translate, rotate and scale specimens to the same size. This allowed us to extract the size component as Centroid Size (Rohlf & Slice, 1990) and to analyse *shape* (form minus size) (Kendall, 1989). This GPA step was used for all specimens as well as subsets (e.g., if only specimens of known sex or mainland-only specimens were considered for corresponding analyses). We included specimens from Groote Eylandt as a separate population for all our analyses, but did not include the specimens from four other island populations for population analyses. This is because of the low sample size for these island populations, as well as the uncertain genetic history (Hohnen et al., 2016; How et al., 2009; Woolley et al., 2015) and possible genetic erosion (Cardoso et al., 2009). We did, however, test whether there were differences in shape variation between island and mainland populations, which would occur if divergent selection on the different islands shaped each population differently.

Morphological differences between populations

In order to explore the shape variation of our dataset, we conducted a Principal Component Analysis (PCA) on the landmark coordinates. This method reduces the large dimensionality of the dataset – due to the large amount of variables (i.e., landmark coordinates) – by tracing orthogonal axes along the main variance-covariance of the data, with the by-product being that the first axis (i.e., principal component) represent most of the shape variation. If population is a determining factor of shape variation in the sample, one of the main Principal Components (PCs) is therefore expected to separate specimens according to populations.

We also assessed for shape differences between populations with Procrustes ANOVAs with permutation tests, and then performed permutation-based pairwise comparisons between the shape and centroid size least squares means of each population (Collyer et al., 2015). Heat plot visualizations of mean comparisons between populations were used to understand where the main shape differences were located. We performed all heat plot visualizations with the *landvR* package (Guillerme & Weisbecker, 2019).

Sexual Dimorphism and Allometry

To assess the degree to which shape variation in the sample was determined by sex differences, we computed the interaction term of Size and Sex on shape to evaluate if both sexes had common allometric relationships. In addition, we corrected for allometric shape differences between sexes by extracting the residuals of allometry of each specimen and adding them up to the consensus shape obtained from the GPA. This allowed us to make heat plots of shape-only sexual dimorphism.

In order to evaluate the influence of size on shape (allometry) in our dataset, we performed a Procrustes ANOVA to quantify the amount of shape variation influenced by the centroid size. We then plotted the Centroid Size vs the projected regression score of shape on size (Drake & Klingenberg, 2008). We used a Homogeneity of Slopes Test to examine if there was a common allometric pattern in all mainland populations plus the Groote Eylandt population.

Association of shape variation with geography, climate and size

To assess the factors influencing cranial shape on the four mainland populations, such as geographical distance among individuals or bioclimatic data, we performed a variation partitioning analysis. For this, we used the *varpart* function in the *vegan* R package version 2.5-6 (Oksanen et al., 2018). Latitude and longitude coordinates of each locality corresponding to each specimen were transformed using a principal coordinates of neighbourhood matrix (PCNM) (Borcard & Legendre, 2002) to avoid spatial autocorrelation. The PCNM method presents several advantages. It produces orthogonal (linearly independent) spatial variables over a much wider range of spatial scales (Pandolfi et al., 2015; Sansalone et al., 2015). Bioclimatic data for all specimens (Annual Mean Temperature, BIO1, and Annual Precipitation, BIO12, of each location) were obtained from WORLDCLIM (v. 2.0) (www.worldclim.org/bioclim) and were included in the final model as a hypothesized influence of a climatic factor on shape. Finally, we performed a redundancy analysis (RDA) on the full model, which includes the three factors (Size, Spatial data and Climatic data) that we hypothesized explained the variation on cranial shape in our study system.

RESULTS

Morphological differences between populations

The Principal Component Analysis reveals no visually obvious shape differentiation among populations along the two main axes of variation. The first two principal components together account for 35% of the total shape variation (PC1 = 24.58%, PC2 = 11.58%) (Supplementary Figure 3). An example of shape changes along the first principal component axis with allometric shape changes put together with other unrelated shape changes is illustrated in Figure 1.

Figure 1 : Shape changes associated with the First Principal Component (above) and with allometry (below). Spheres are the landmarks used in this study. Warmer colours represent higher landmark variation between mean shapes. Vectors show direction and magnitude of shape variation.

Despite the low differentiation of populations within a PCA, the Procrustes ANOVA demonstrates that at least one of the five populations differ in shape ($F_{(4,91)} = 3.125$, R-squared = 0.121, $p < 0.001$) (Table 1). The post-hoc pairwise comparisons between the shape means of each population reveal significant differences in shape among all populations. Intriguingly, the only sex-biased population (Kimberley, which consisted mostly of males) shows no clear difference with the remaining four populations (Figure 2). Groote Eylandt specimens show a generally narrower skull as revealed by the greater interlandmark distances in the zygomatic arches. The four mainland populations have shorter muzzles than the Groote specimens, as revealed by the shortening of the nasal and frontal areas. Northern Territory specimens display elongated frontal bones. Pilbara specimens exhibit an expansion of the braincase size and shortest muzzles relative to the rest of the skull. Shape disparity (shape variance) differences between island and mainland specimens were not significant ($p = 0.604$).

Figure 2 : Pairwise comparisons between means of each population and visualization of interlandmark variation between populations mean shapes. Warmer colours represent higher landmark variation between mean shapes. Top left, comparisons of absolute mean shape of each population; bottom right, comparisons of size-corrected mean shapes of each population. Map on bottom left shows all specimen locations used in this study.

Sexual dimorphism and allometry

We first confirmed that known sexual dimorphism in animal weight and skeletal measurements (Oakwood, 1997; Schmitt et al., 1989) are reflected in cranial size (Supplementary Figure 4) and shape (Table 1 and Supplementary Figure 5). Size differences are significant between males and females ($F_{(1,90)} = 23.9$, R-squared = 0.21, $p < 0.001$), island and mainland populations ($F_{(1,99)} = 14.15$, R-squared = 0.125, $p < 0.001$) and populations ($F_{(4,91)} = 8.361$, R-squared = 0.269, $p < 0.001$).

Females and males show the same homogenous allometric relationship with no significant difference between

slopes ($p = 0.087$), such that small males and large females overlap on the allometric slope (see Supplementary Figure 6). Just over half of sexual shape differences are due to size (R-squared for shape between sexes = 0.102), with a component of shape changes not due to size (R-squared of allometry-adjusted shape differences between sexes = 0.043) (Table 1). In other words, although there is some non-size-related variation between sexes, small males and large females are similarly shaped according to their common size. We therefore included individuals of both sexes in our analyses of population differences and other pertinent analyses.

In the full dataset, and among all variables tested, size manifests as the strongest determinant of shape variation in northern quolls ($p < 0.001$), accounting for 15.1 % of the total shape variation. A Homogeneity of Slopes Test suggests no significant differences among allometric slopes of each population ($p = 0.203$) (Table 1 and Figure 3), meaning that the hypothesis of populations following the same allometric slope is not rejected. Allometry-corrected shape analysis also reveals the shape differences between populations; Procrustes ANOVA performed on the residuals of allometry revealed significant differences between populations ($F_{(4,91)} = 3.419$, R-squared = 0.131, $p < 0.001$). Pairwise comparisons between these size-corrected shapes show similar significant differences between populations. Thus allometry does not play a role in differentiating the shape of populations.

Figure 3 : Allometry plot, centroid sizes (proxy for body size) versus shape scores obtained from the regression of shape on size (Drake & Klingenberg, 2008). Results of Homogeneity of Slopes Test for allometric slopes of populations are shown on Table 1.

Association of shape variation with geography, climate and size

Shape differences show significant differences along both latitudinal ($F_{(1,87)} = 4.051$, R-squared = 0.044, $p = 0.002$) and longitudinal ($F_{(1,87)} = 3.023$, R-squared = 0.034, $p = 0.003$) gradients on mainland specimens. Size is significantly different along the latitudinal gradient ($F_{(1,87)} = 8.833$, R-squared = 0.092, $p = 0.004$; but not along the longitudinal gradient, $p = 0.117$). Temperature and precipitation contribute small, but significant, effects to shape (Temperature: $F_{(1,87)} = 2.006$, R-squared = 0.023, $p = 0.029$; Precipitation: $F_{(1,87)} = 3.411$, R-squared = 0.038, $p = 0.002$). Size differences are only significantly predicted by the effect of precipitation ($F_{(1,87)} = 8.236$, R-squared = 0.086, $p = 0.005$), but not by the effect of temperature ($p = 0.794$).

We dissected the influence of size, geography and climate (precipitation + temperature) with a variation partitioning analysis (Figure 4). The full model [a + b + c + d + e + f + g] shows a significant effect of these three factors on cranial shape variation ($F_{(13,75)} = 3.136$, adjusted R-squared = 0.24, $p < 0.001$). Climatic variables alone [c] do not explain any of the variation ($p = 0.224$), however, they contribute to the model when geography is considered jointly [f] (adjusted R-squared = 0.05). Pure geographical distances [a] explain 3 % (adjusted R-squared = 0.033) of the shape variation ($F_{(10,75)} = 1.371$, $p = 0.004$). Finally, in accordance with our predictions, size alone [b] contributes mostly to the model by accounting for 17 % of the total cranial shape variation ($F_{(1,75)} = 17.539$, adjusted R-squared = 0.165, $p < 0.001$).

Figure 4 : Schematic representation of the variation partitioning analysis (VARPART), which included effect of geography, size and combined climatic variables (precipitation and temperature) on cranial shape. The values shown in the diagram represent the individual fractions for each set. The outer numbers are the adjusted R-squared values of pure geography [a], pure size [b] and pure climate [c] and the inner values are the adjusted R-squared values of the interaction of the corresponding explanatory variables. The individual fraction for the interaction of all three variables [g] is negligible and not shown. The amount of unexplained shape by this model is depicted by the residuals (76%). Circle sizes are schematic and do not represent the amount of shape explained by the model.

DISCUSSION

The rationale of this study was the expectation that the biogeographic adaptive and genetic divergences between northern quolls should be evident across their 5000-kilometre longitudinal range. Our aim was to improve our understanding on whether the species represent morphological or functional conservation

units, which could be used alongside population genetic approaches in the conservation management of the species. Surprisingly, however, we found little structure in northern quoll shape variation (~76% of shape variation remains unexplained) and no strong evidence that any of the populations have evolved into discrete, possibly locally adapted, morphotypes. In particular, populations and sex differences have low effect sizes and explain less variation than size, meaning that similarly-sized individuals from opposite ends of the biogeographic distribution are likely to be similar in shape, even if they are of different sex. It also seems that most variation is evenly distributed within each of the populations, such that more localized western populations appear just as disparate in shape as individuals across the length of the eastern Queensland seaboard.

There is some limited support for our concern that developmental constraints might reduce the adaptability of northern quoll skulls, as size explains the most shape variation and the populations seem to differentiate by size. However, given the low amount of variation (~16%) that size explains, and the broad overlap of populations in size and shape, any such constraint is unlikely to be strong. However, it is an intriguing indication that at least some of the larger-scale evolutionary association between skull shape and size among marsupials may be visible at the within-species level. This is contrary to findings in other marsupials (Mitchell, Sherratt, Sansalone, et al., 2018; Weisbecker et al., 2019), and might represent one of several ways to shape morphological traits within the species.

Although differentiation of skull shape between populations had low effect sizes, populations and sex are statistically distinguishable, even when size is taken into account. This might suggest a stochastic, possibly heritable, shallow divergence between populations which, however, does not appear to reflect local adaptation. These effects also demonstrate the ability of skull shape to vary independently of size based on genetic factors related to sex or population, again contradicting the developmental constraints hypothesis. Thus, the population divergences do not appear to coincide with adaptive morphological differentiations. This provides an indication that genetic fitness benefits of outbreeding populations (Cardoso et al., 2009; Kelly & Phillips, 2019) would not risk any adverse effect due to differential local adaptation, although of course this would need to be further investigated based on non-morphological (behavioural or physiological) traits.

To better understand the functional implications of shape divergence among the northern quolls, the displacement between landmark configurations according to size, population, and sex can be visually interpreted. The landmark displacement predicted by allometry identifies two main regions of variation: larger skulls tend to have overall smaller braincases relative to the rest of the skull, a larger sagittal crest, a more anteriorly positioned masseteric scar and associated dorsally-oriented zygomatic arch. Differences between sexes include males with larger sagittal crests, smaller braincases, shorter nasals and wider zygomatic arches.

Rearrangements of the zygomatic arch and the muzzle appear to distinguish some northern quoll populations. This variation mirrors a well-known evolutionary adaptation to changes in mastication (Meloro, 2011; Mitchell, Sherratt, Sansalone, et al., 2018; Mitchell & Wroe, 2019; Weisbecker et al., 2019). It is therefore possible that the variation in muzzle length and zygomatic arch placement between some populations could be interpreted as adaptation to a particular diet or feeding habit within each population. For instance, populations in drier environments, such as Pilbara or Groote Eylandt, show a shortening in the muzzle, when compared to wetter environments, such as Northern Territory or Queensland. Shorter faces are known to imply greater bite forces, and thus might indicate the mastication of tougher foods consisting of more vertebrates (Wroe & Milne, 2007). However, whether this is truly an adaptive effect is doubtful due to the abovementioned low effect sizes and extensive overlap in shape between populations, as well as the lack of association between climatic factors and shape. In addition, although precipitation is a main predictor of quoll diets (Fancourt et al., 2015), this variable explains little variation in shape.

Aside from hypotheses of microevolutionary adaptation, it is also possible that much of the variation in the northern quoll skull derives from a re-modelling process based on individual uses of the skull. Perhaps the best example of this is the sagittal crest, which varies widely in length among northern quoll individuals. It is common for mammals – particularly males – to display larger sagittal crests with age (Flores et al., 2006), but this is a purely behavioural consequence of the pulling action of the temporalis muscle (Washburn,

1947). Similarly, bone re-modelling during an individual's lifetime has been suggested for the zygomatic arch of mammals (Abdala & Giannini, 2000; Ravosa, 1991), and is also suspected in wombats and kangaroos (Mitchell, Sherratt, Ledogar, et al., 2018; Mitchell, Sherratt, Sansalone, et al., 2018; Weisbecker et al., 2019). It is therefore possible that the emphasis of shape variation on the masticatory apparatus, found along PC1 and the allometric pattern, arise from masticatory behaviours and possibly the “mating bite” (Braithwaite & Griffiths, 1994; Oakwood, 2000) of the larger males. This is also consistent with the weak tendency of males to have shorter muzzles than females, regardless of size, as shorter muzzles are related to higher bite forces (Wroe & Milne, 2007). This again suggests that the differences between populations are not a decisive factor to the individuals' survival, and rather originate from potentially non-adaptive factors such as genetic drift or individual variation in feeding (Weisbecker et al., 2019) between populations.

The geometric morphometric analyses of northern quoll skull shape adds useful, quantitative, phenomic data to assessments of variation across the distribution of an endangered marsupial. The overarching find of low morphological differentiation, and very high levels of unexplained variation, has two important implications. First, it suggests that individuals of different populations are not locally adapted to the point where a separation of population phenomes is indicated (although it needs to be investigated if there might be behavioural or physiological reasons to do so). On the other hand, the lack of differentiation across the diversity of biomes, climatic conditions, or populations is a concern because it suggests a low adaptability of the species to environmental change. The concentration of shape variation in the masticatory apparatus suggests individual plasticity is a major response mechanism in the determination of northern quoll skull shape, suggesting that there is little scope for larger-scale, heritable variation within the species. A similar pattern of potentially high within-species plasticity in the masticatory apparatus has also been suggested for the living wombat species as well as kangaroos; together, the concerning suggestion is that marsupial mammals might have a scope for individual plasticity, but not evolve specific adaptations within short time spans. Further research should be directed into identifying the scope of shape variation in other threatened marsupials, investigating other climatic variables or patterns as predictors, and adding biomechanical and developmental studies to further dissect the variation that exists in this clade; in addition, a comparison with ecologically similar placental species would be useful to identify if marsupials show less intrinsic capacity of shape variation than placental mammals.

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v. DATA AVAILABILITY STATEMENT

Data is publicly available in Dryad. R code is also publicly available in GitHub. 3D models can be publicly accessed through Morphosource.

Table 1 : Results of Procrustes ANOVA on factors of shape variation.

Factors	Formula	Question
Populations (four mainland + one island, Grootte Eylandt)	Shape~population	Are there population differences in shape?
Size (Allometry)	Shape~size	Is there allometry?
Size:Sex (only reporting interaction term significance)	Shape ~ size*sex	As there is sexual dimorphism and allometry, is there a size*sex interaction?
Size-corrected sex differences	Shape~size+sex	Adjusting for size, is there sexual dimorphism?
Sex (shape)	Shape~sex	Are there sex differences in shape?
Size:Population (Homogeneity of Allometric Slopes)	Shape~size*population	Do populations share a common allometry?
Island / Mainland	Shape~island/mainland	Are there differences in shape between island and mainland?

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NquollShapeVariation_Figures_Blinded.pptx available at <https://authorea.com/users/302259/articles/432291-skull-shape-of-a-widely-distributed-endangered-marsupial-reveals-little-evidence-of-local-adaptation-between-fragmented-populations>