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Assessing sex ratio and density of two koala populations on the North Coast of New South Wales from genetic analysis of scats

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Executive summary

It is notoriously difficult to estimate the size of animal populations, yet it is fundamental for understanding their ecology and improving management outcomes. This is particularly so for threatened species, such as the koala (*Phascolarctos cinereus*) that can be difficult to detect with many survey methods in forested landscapes. Recent advances with remote acoustic sensors and modelling of unmarked individuals has allowed assessments to be made of the density of koalas. However, this approach requires assumptions to be made about the sex ratio of populations since it is primarily male koalas that are detected using acoustics. We applied a complementary approach to assess sex ratio and koala density using microsatellite markers and genetic sex determination of koala scats at two sites on the North Coast of New South Wales for which independent estimates of koala density were obtained using acoustics. Genetic sex-typing revealed that the ratio of male-to-female koalas did not differ from parity, though sample sizes were relatively small at both sites (n=8 and n=26 individuals based on genotyping). Analysis of the microsatellite markers provided crude density estimates that were broadly comparable to the estimates from acoustics. Both approaches indicated the presence of a low-density population (<0.1 koalas ha^{-1}) at each site. Additionally, the microsatellite data revealed that proximate males but not females were genetically similar, which could indicate female biased-dispersal and extends the value of this approach beyond assessing koala density.

Introduction

It is notoriously difficult to estimate the size of animal populations, yet it is fundamental for understanding their ecology and improving management outcomes. This is particularly so for threatened species, such as the koala (*Phascolarctos cinereus*) that can occur in low numbers and are difficult to detect with many survey methods in forested landscapes (4-24 % detection), including call playback and spotlight surveys (Kavanagh and Stanton 1995; Kavanagh et al.1995; Smith et al.1994).

Recent advances with remote acoustic sensors coupled with software that can recognise species-specific calls have provided a robust and cost-effective method to detect koala populations in forestry landscapes, with rates of detection as high as 43 % per night of sampling (Law et al. 2018). Such methods are ideal for monitoring trends in site occupancy, but current technology does not differentiate bellows made by different individuals which is a requirement of traditional mark-recapture, and more sophisticated spatially explicit capture recapture (SECR) techniques used to estimate density.

Developments in data analysis (Spatial Count Models – Chandler and Royle 2013) suggest it is also possible to monitor changes in the density of species when individuals are not recognised (i.e. unmarked), such as from an array of acoustic sensors. This approach also accounts for imperfect detection which avoids underestimates of density. Most importantly, all estimates of density are bounded by confidence intervals. Furthermore, this method has been tested for koalas at a range of forest sites in NSW (Law et al. in press). However, one of the limitations of this approach is that it is primarily male koalas that are detected, so to transfer these data to whole adult population estimates, assumptions need to be made about sex ratio. While estimates in the literature indicate koala populations typically occur in a 1:1 ratio (Martin 1985; Penn et al. 2000; Watchorn and Whisson 2019), additional assessments in areas of interest remain valuable.

Genetic assessment of koala scats may also allow for koala density to be estimated (Wedrowicz et al. 2013). Coupled with spatially-explicit-capture-recapture (SECR) modelling, the technique has the potential to provide density estimates and also measures of uncertainty. When sample sizes are low or recaptures are not sufficient for SECR modelling, the technique may still be used to provide a crude density estimate for a designated search area. Furthermore, additional information can be obtained, including the sex of each individual.

To complement estimates of density using acoustics, genetic assessment of koala scats (following Wedrowicz et al. 2013) was undertaken at two sites (Kalateenee State forest, 2019, pre-harvest; and Kiwarrak State forest, 2020, one-year post-fire) to identify the sex ratio of the population and the number of unique individuals. The aims of this study were to: 1) report on sex ratio of koala populations at each site and 2) provide crude estimates of koala density that could be compared with independent estimates derived using acoustic arrays.

Methods

Acoustic arrays covering an area of 400 ha each in Kalateenee State forest and Kiwarrak State forest were surveyed in October 2019 and August 2020 (post-2019 bushfire), respectively, using remote sound recorders (SM4) to detect male koala bellows and subsequently model male koala density (Fig. 1 and Fig. 2). Sound recorders sampled for a period of 10 nights (sunset-sunrise). For more details, see Law et al. (in press).

At Kalateenee State forest, three pre-dawn drone flights were undertaken to detect live koalas, and to identify areas to search using koala scat detection dogs (A. Roff unpubl. data). Drone flights covered ~60 ha of the grid per night (Fig. 1a). Koala scat detection dogs were used to search a subset of the grid (14 grid cells; 224 ha) as the grid was too large for an entire search and topography or dense vegetation impeded movements by dogs. The search area each day was guided by the location of drone hotspot detections but also covered additional areas not surveyed by the drone or areas without drone hotspot detections. Koala scats detected by detection dogs were collected (old scats were not collected), with 1-4 pellets collected from 48 locations within the acoustic grid. In all, 90 scats were collected for downstream genetic analysis (Fig. 1b; sex-typing and genotyping; see Wedrowicz et al. 2013). An additional six samples from three GPS-collared koalas with home ranges that encompassed or were adjacent to the acoustic grid were also included in analyses. These were used to confirm the reliability of the analysis.

At the second acoustic grid site in Kiwarrak State forest, 131 scats were collected from 58 locations. A drone wasn't available during this survey, so the search area covered different parts of the grid using koala scat detection dogs over three days (13 grid cells; 208 ha). However, given the topography of the site, searching was limited to the more accessible parts of the grid and where vegetation was not dense, which was a major issue at this site one year after a significant fire (Hillville mega-fire; Fig. 2b).

Molecular techniques

DNA Extraction

Deoxyribonucleic acid (DNA) was extracted from surface washes of faecal pellets following the method of Wedrowicz et al. (2013). Each pellet was placed in 3 mL of 1% phosphate buffered saline and gently rotated for 15 minutes on a rotatory mixer. The pellet was then removed, placed into a labelled zip lock bag and stored at -20℃. The phosphate buffered saline wash was centrifuged for 15 minutes at 4 000 g and the supernatant removed. DNA was extracted from the pellet wash by Digsol/proteinase K digestion, following the method of Bruford et al. (1998) and eluted in 50 uL of molecular grade nuclease free water (Ambion). Polymerase chain reaction (PCR) inhibitors were removed from the extracted DNA using a OneStep PCR inhibitor removal kit (Zymo) following the manufacturer's instructions.

Sex-typing

A scat was identified as from a male koala if a fragment of the SRY gene could be amplified from the faecal wash DNA by polymerase chain reaction (PCR) using primers designed by Wedrowicz et. al. (2018) for Queensland koalas (PCY-F: 5′-TCTGGAGAATCCCAAAATGC-3′ and PCY-R: 5′-ATTCTTCCCTGTGTTTAGCG-3′). Following Wedrowicz et al. (2013) a fragment of the Glucose-6-phosphate dehydrogenase (GPD) gene was also amplified from the faecal wash DNA as an internal positive control using the following primers: GpdEx12: CTCTCCCTCTAGTGATGAGC and GpdEx13R: CCACTTGTAGGTGCCCTCATACTGGAA (Loebel and Johnston 1997; Loebel et al. 1995). If only the GPD band amplified, then the scat was identified as from a female. Scats were identified as from a male where both the GPD and SRY fragments amplified. If neither fragment amplified or only the SRY fragment amplified, then the sex was designated as unidentified.

Each PCR (20 ml) contained 2 µL of DNA template, 1 x MyTaq Red PCR reaction mix (Bioline), each SRY primer at 0.1 µM, each GPD primer at 0.15 µM and 0.05 µL of bovine

serum albumin (20 mg/ml). Amplification conditions were as follows: 3 min initial denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, annealing for 30 s at 58°C and extension for 30 s at 72°C; with a final extension of 4 min at 72°C. The SRY and GPB PCR products were separated and visualised on 2% agarose gels in 1-Tris-borate-EDTA (TBE).

Scats were identified as being produced by a male koala if a fragment of the SRY gene could be amplified from the pellet wash DNA by PCR, using primers designed by Wedrowicz et. al. (2018) for Queensland koalas (PCY-F: 5′-TCTGGAGAATCCCAAAATGC-3′ and PCY-R: 5′-ATTCTTCCCTGTGTTTAGCG-3′). Following the method developed by Wedrowicz et al. (2013), a fragment of the Glucose-6-phosphate dehydrogenase (GPD) gene was also amplified from the pellet wash DNA as an internal positive control using the primers developed by Loebel and Johnston (1997), Loebel et al. (1995 GpdEx12: CTCTCCCTCTAGTGATGAGC and GpdEx13R: CCACTTGTAGGTGCCCTCATACTGGAA). If only the GPD band amplified, the pellet was identified as being produced by a female koala. Scats were identified as from a male where both the GPD and SRY fragments amplified. If neither fragment amplified, or only the SRY fragment amplified, sex was designated as undetermined.

PCR reactions consisted of 1 x MyTaq Red PCR reaction mix (Bioline), 0.1 µM of the SRY forward and reverse primers, 0.15 µM of the GPD forward and reverse primers, 2 µl of DNA template, and 0.05 µl of bovine serum albumin. Reaction volumes were adjusted to 10 µl using nuclease free water. Amplification conditions were: 3 min initial denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, annealing for 30 s at 58°C and extension for 30 s at 72°C; with a final extension of 4 min at 72°C. The SRY and GPB PCR products were separated by electrophoresis and visualised on 2% agarose gels.

Microsatellite Genotyping

All scats collected from Kalateenee for which the GPD fragment successfully amplified, and an additional three samples that had high DNA concentrations, were screened in triplicate for a panel of 31 microsatellite markers. The panel was amplified in three multiplex assays, with modification of some of the published primers to adjust the allele size ranges (L. Hulse – University of Queensland, unpublished data). All forward primers incorporated a fluorescent label. Amplification conditions were: initial denaturation at 94 °C for 10 minutes, followed by 12 cycles of 94 °C for 30 seconds, 65 °C to 52 °C (stepped down 1 °C each cycle) for 45 seconds and 72 °C for 60 seconds, and then 30 cycles of 94 °C for 30 seconds, 54 °C for 45 seconds and 72 °C for 60 seconds. Alleles were separated by capillary electrophoresis at the Australian Genome Research Facility and scored with GeneMarker V2.7.0 (SoftGenetics, PA, USA). After examination of the traces, eleven of the 31 markers Phc4 and Phc11 (Houlden et al. 1996); Phci2, Phici9, Phci27 and Phci5 (Ruiz-Rodriguez et al. 2014); Pcin20, Pcin23, Pcin03, Pcin05 and Pcin10 (Dennison et al. 2017) were found to reliably amplify without stutter peaks, or off sequence alleles, and generally returned consistent results among replicates. This conservative panel of eleven markers was retained for further analysis.

The findings of the initial microsatellite analysis of the Kalateenee samples were confirmed and the genotyping of samples from Kiwarrak was performed following the protocol of Wedrowicz et al. (2017), using a panel of 12 microsatellite markers: K2.1, K10.1, Pcv6.1, Pcv6.3, Pcv24.2, Pvc25.2, Pcv30, and Pcv31 (Critescu et al. 2009); Phc4 and Phc13 (Houlden et al. 1996); Phci2 and Phci10 (Ruiz-Rodriguez et al. 2014). A single scat sample from each sampling point from the Kalateenee (with the exception of eight samples for which no DNA remained) and Kiwarrak search areas, for which the GPD fragment successfully amplified, was genotyped in triplicate, with low DNA concentration samples run a fourth time.

Molecular analysis

Identification of Individuals

Putative microsatellite genotype matches and near matches were identified in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Two samples were considered to be from the same individual if their profiles matched or any mismatches could be attributed to allele dropout (one of the samples was homozygous for an allele present in the other sample), or the mismatching locus was only able to be scored for a single replicate suggesting low DNA quality and high error rate. For the Kalateenee samples, individuals were identified from their combined genotype across the two marker panels.

Spatial genetic structure within sites

Spatial genetic autocorrelation analysis was conducted for male and female Kalateenee samples both separately and combined using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Each identified individual was only included in the analysis once (even if multiple samples were identified as the same individual). Pairwise genetic distances were calculated using the method of Smouse and Peakall (1999), using the combined microsatellite loci from the two panels (after removal of the two duplicate loci). The geographic distance between pair members was calculated from the GPS coordinates where the samples were found. If scats from multiple locations were identified as the same individual, the average of the latitude and longitude values for the different locations were taken as the koala's location. A distance class size of 200 m with a maximum distance class of 1000 m was used. Autocorrelation r values were deemed significantly different from zero if the 95 % Confidence interval as determined by bootstrapping (n =1000), did not overlap zero. Bootstrapping was only performed when there were greater than 5 comparisons within a distance class.

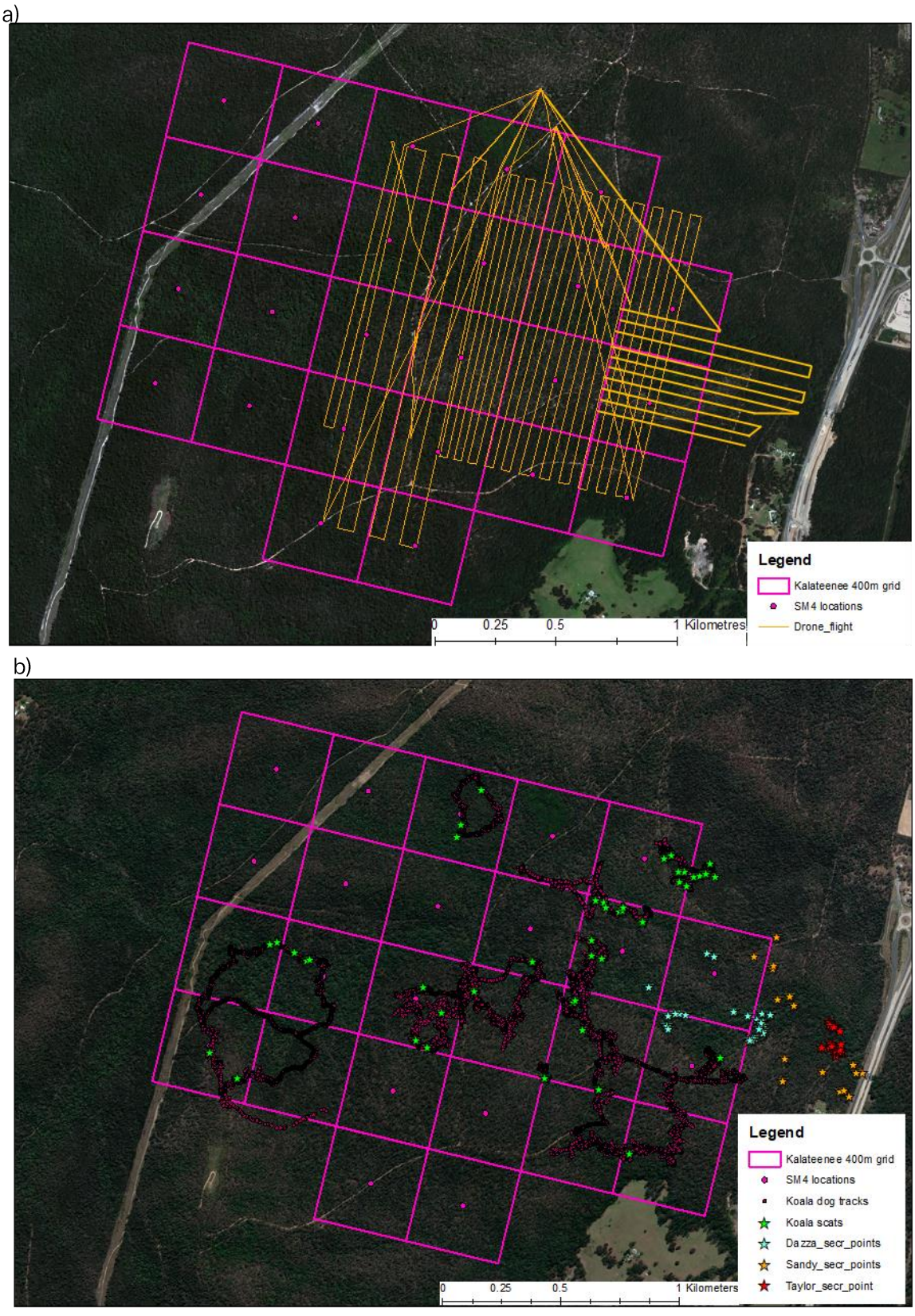

Fig. 1. Satellite image of the Kalateenee State forest area illustrating: a) areas searched for koalas with a thermal drone and b) koala detection dog tracks and locations of sampled scats for genetic analysis. The locations of three GPS collared koalas (Dazza, Sandy and Taylor) are also shown for the days of sampling with detection dogs.

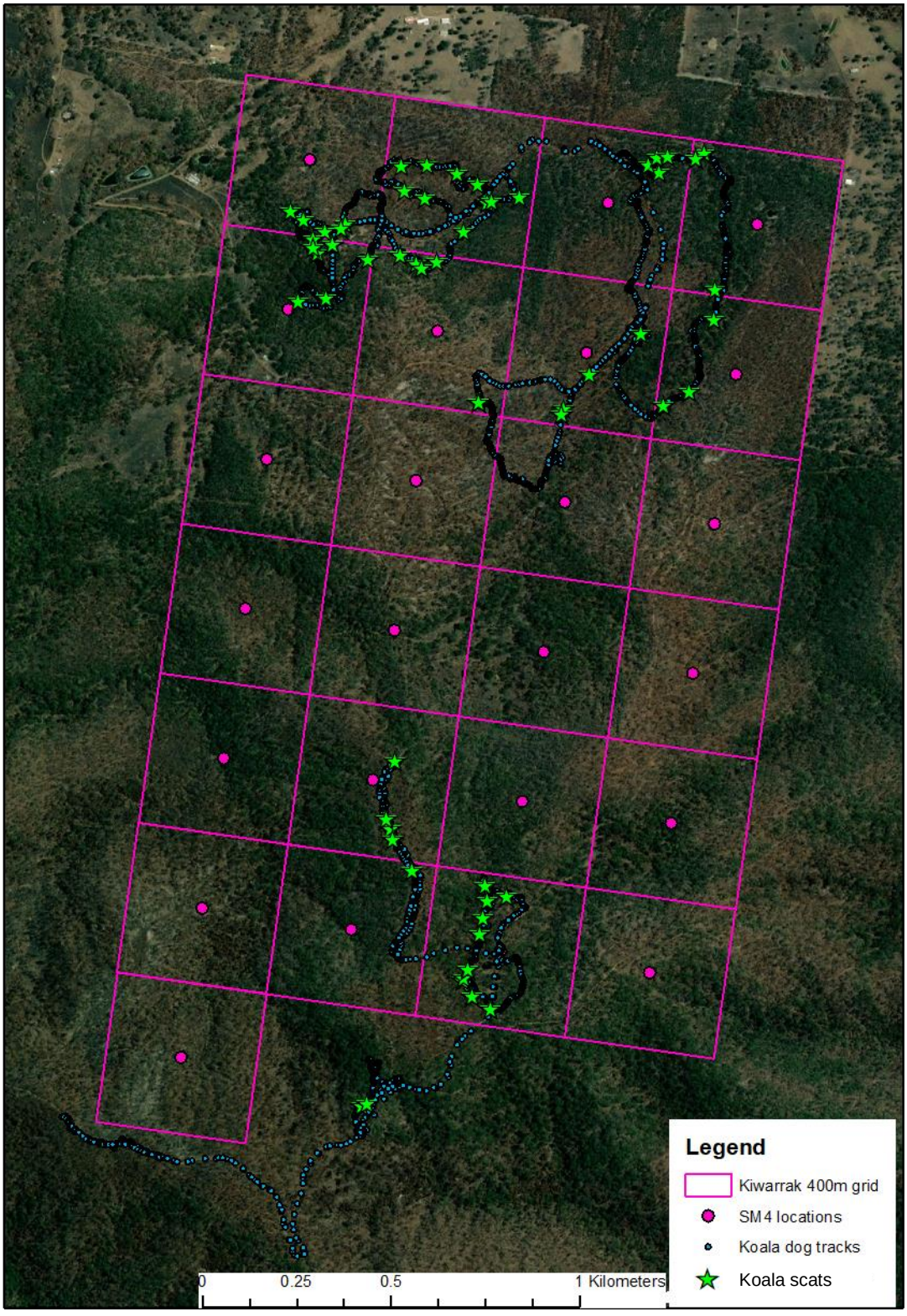

Fig. 2. Satellite image of the Kiwarrak State forest study area illustrating location of acoustic array points (SM4 points), koala detection dog tracks and locations of sampled scats for genetic analysis.

Results

Kalateenee

Of the 90 scat samples collected from the Kalateenee search area the koala's sex could be identified for 73 (81.1%), with 30 samples from female koalas and 43 from male koalas (58.9% males). Notably, only males were detected at the south-eastern end of the site (Fig. 3). The sex was determined for all six samples from the three known koalas and matched the koalas' actual sex.

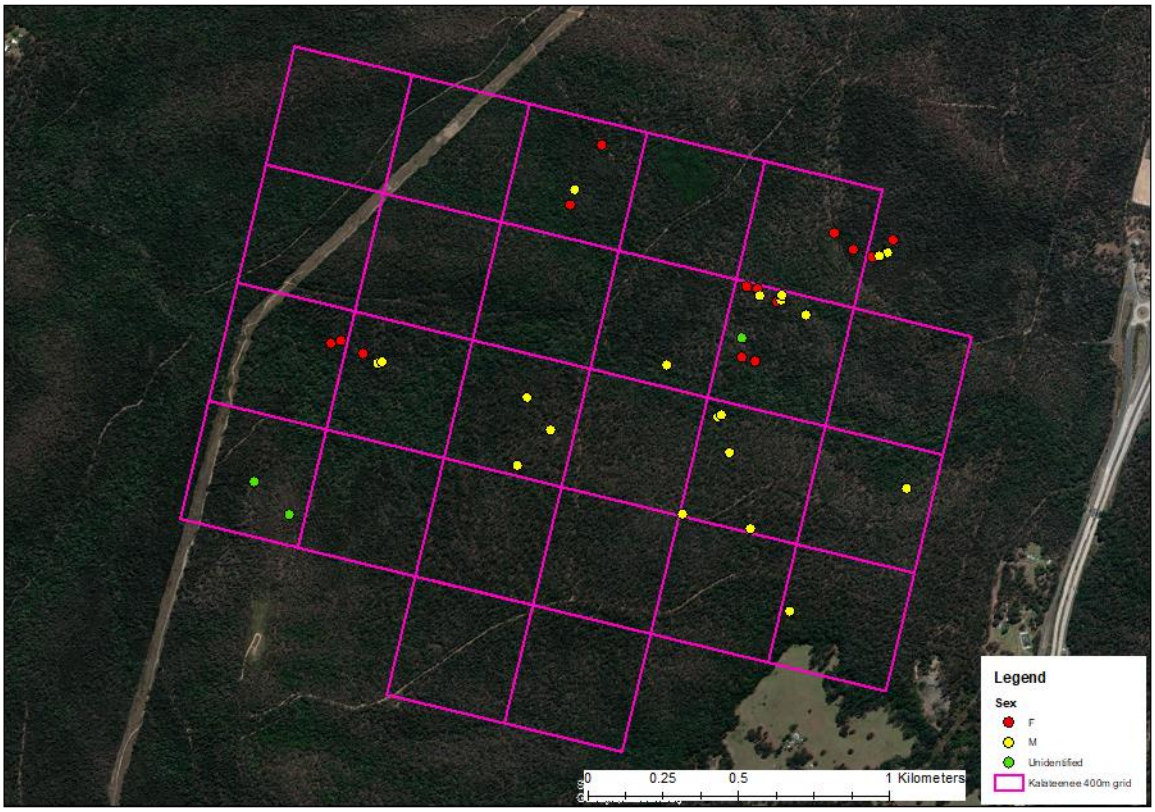

Fig. 3. Distribution of female and male koala scats in the Kalateenee State forest study area. Note that multiple scat locations can be from the same individual.

Microsatellite genotypes were obtained using the first panel of markers for 76 samples from 42 of the 48 sampling locations. All samples from the same sampling point had matching genotypes and were therefore likely to be from the same individual. Profiles were then successfully amplified for 31 of the 42 sampling points using the second

microsatellite panel. In general, the designations of unique individuals were in agreement between the two marker panels. The single exception was a group of six samples that were identified as from two individuals using the first panel and four individuals using the second panel. By combining the profiles from both panels these six samples were identified as from five individuals. From the combined genotypes, a total of 26 individuals were identified from the 31 sampling points (Fig. 4). Of these, ten were female, 13 were male and three were of unknown sex (56.5% males), indicating no significant difference from a 1:1 ratio (chi-square=0.391, p=0.531). A single sample from the eastern extent of the site matched one of the known koalas (Dazza, Fig. 4). This sample was collected from within Dazza's home range as determined from GPS and VHF radio tracking.

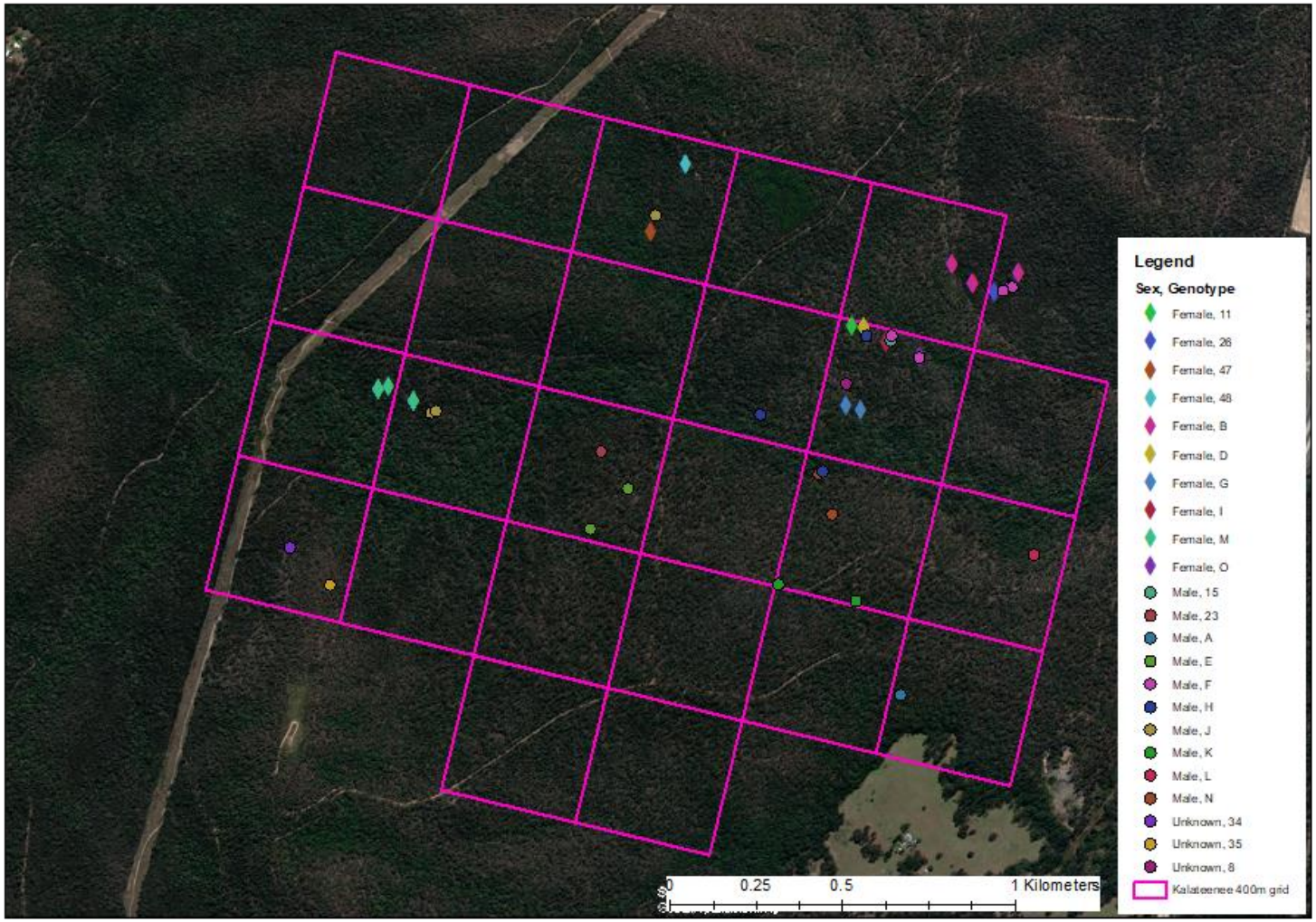

Fig. 4. Distribution of unique individuals in the Kalateenee SF study area. Scats belonging to female and male koalas are denoted by diamonds and circles, respectively. Scat belonging to collared koala 'Dazza' is labelled as 'Male L'.

The spatial genetic autocorrelation analysis revealed no significant genetic structure among males and females combined over the Kalateenee search area (Fig. 5a). There was also no significant spatial genetic structure among female koalas (Fig. 5b). However, there was spatial genetic structure among male koalas, with koalas within 400 m from one another more genetically similar than males that were further apart (Fig. 5c). This suggests that male koalas within 400 m of one another were more likely to be close relatives.

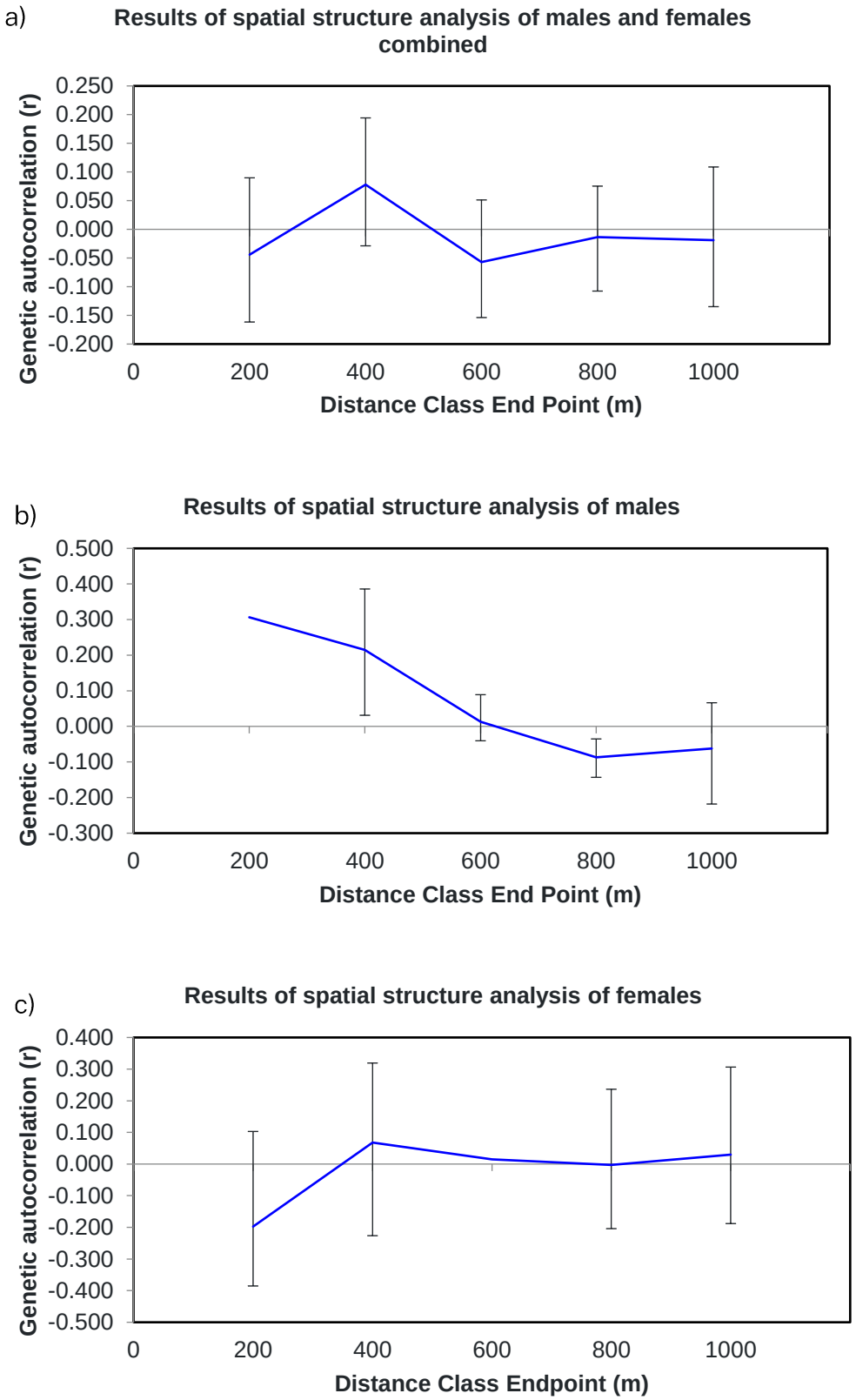

Fig. 5. Correlogram of genetic autocorrelation r by geographic distance for males and females combined (a), as well as for males (b) and females (c) separately.

Kiwarrak

Of the 131 scat samples collected from the Kiwarrak search area, koala sex was identified for 39 (29.8%) scats from 26 of the 58 locations, with 19 samples from female koalas and 20 from male koalas (51.3% males; Fig. 6). Notably, there were four cases where two samples from the same sampling point were assigned to different sexes. It is unknown whether this was due to drop-out of the SRY gene due to low DNA concentration or if the scats at those sampling locations were produced by two koalas. In each instance, the "female" sample was microsatellite genotyped.

Genotypes for at least 11 of the 12 microsatellites were obtained for 12 samples from 11 of the 26 sampling locations. Eight individuals were identified from these 12 samples, three females and five males (Fig. 7). While the number of individuals identified for this site was very low, the sex-typing results suggest that the sex-ratio was not substantially male or female biased (Fisher's exact test 2-tail; p=1).

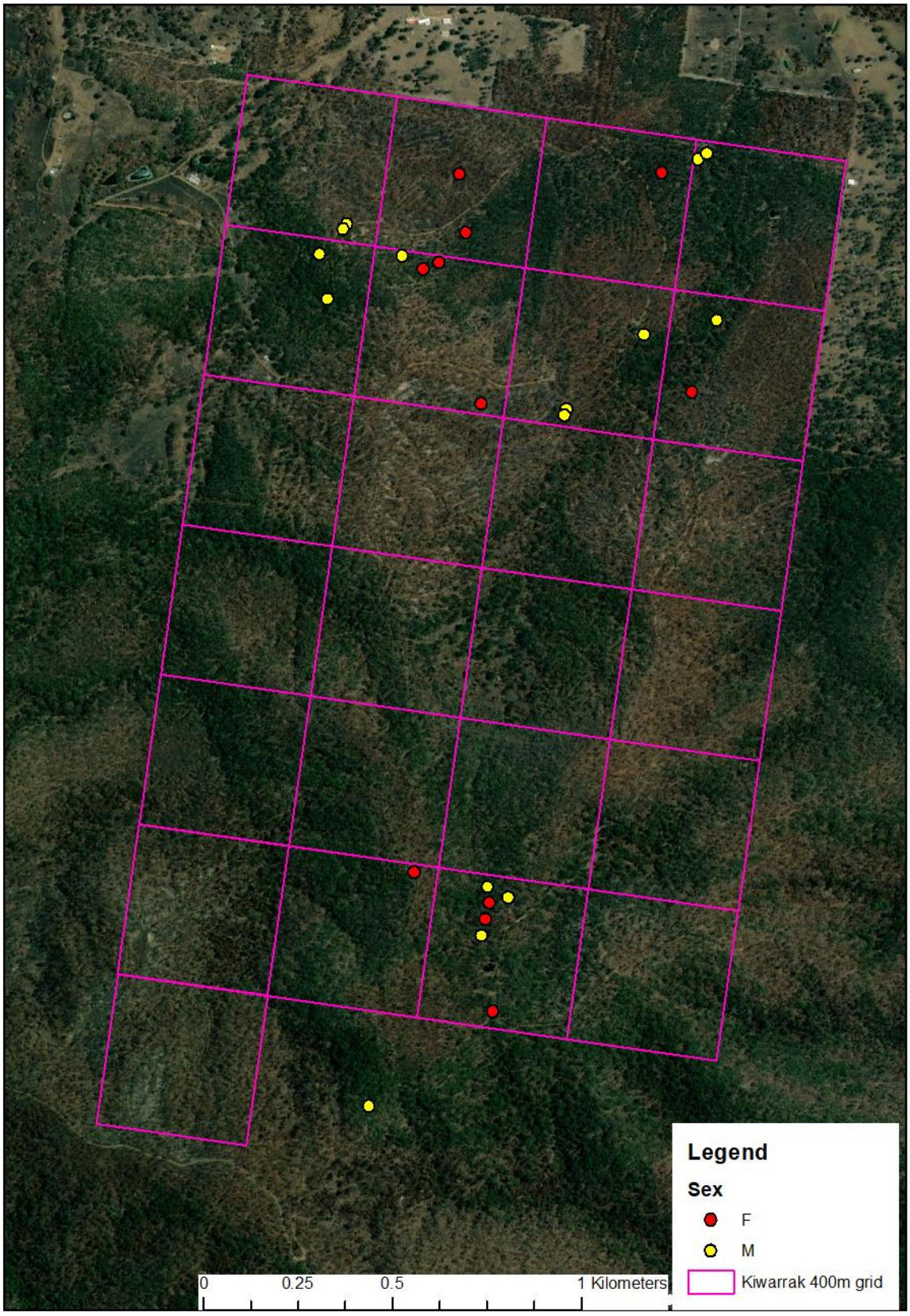

Fig. 6. Distribution of female and male koala scats in the Kiwarrak State forest study area. Note multiple scat locations can be from the same individual.

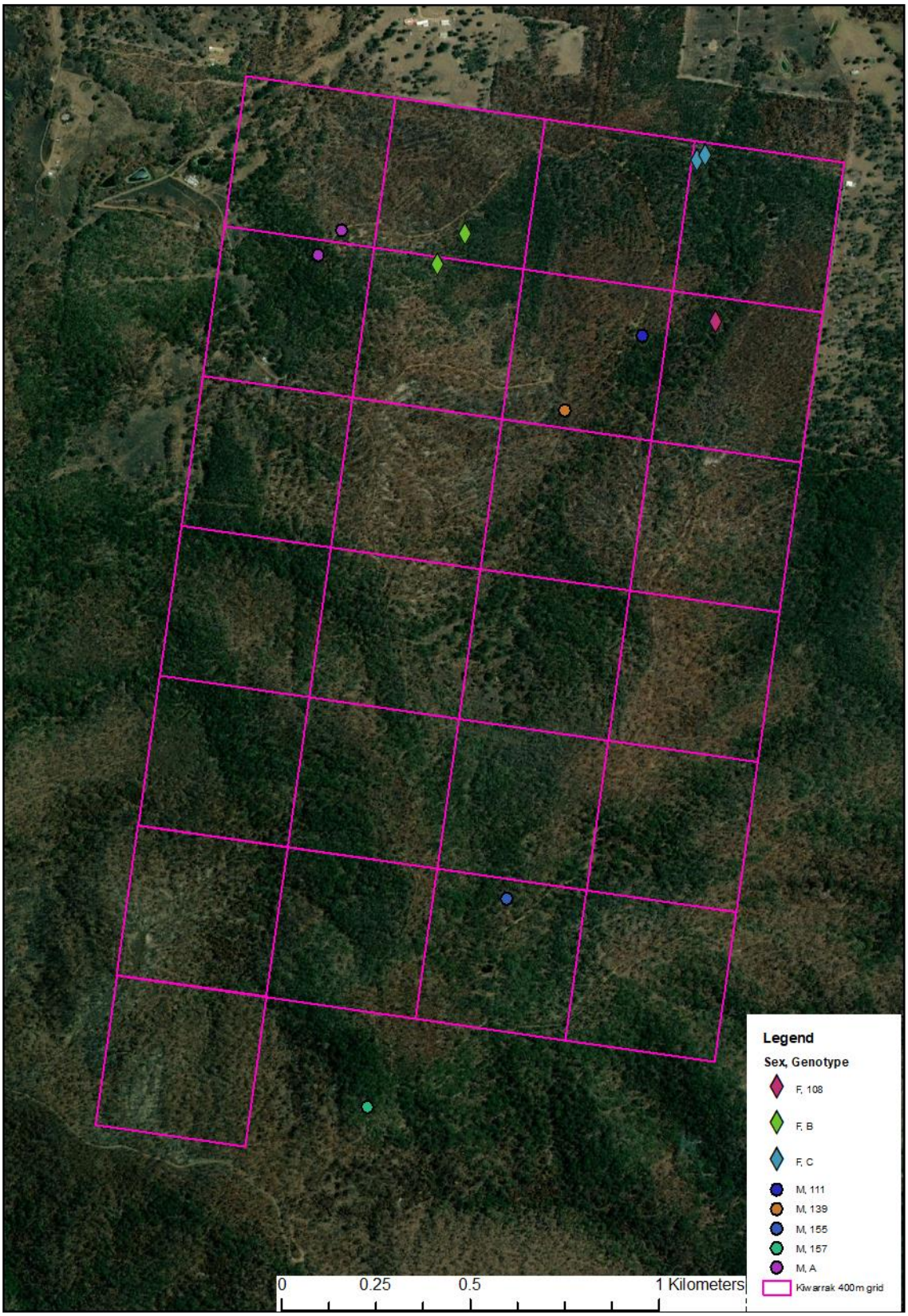
Legend
Sex, Genotype
F, 108
F, B
F, C
M, 111
M, 139
M, 155
M, 157
M, A
Kiwarrak 400m grid
0
0.25
0.5
1 Kilometers

Fig. 7. Satellite image of the Kiwarrak State forest study area, demonstrating the distribution of unique individuals within the study area. Note, scats belonging to female and male koalas are denoted by diamonds and circles, respectively.

Koala density estimates

Koala density estimates obtained from acoustic sampling were broadly consistent with crude estimates obtained from genetic sampling, though the scat-based estimate for Kiwarrak is likely to be an underestimate given the low number of samples successfully genotyped. At Kalateenee, a crude density estimate of 0.1 koalas ha^{-1} was calculated for a search area of 224 ha (56 % of array). This was slightly higher than the acoustic estimate of 0.07 koalas ha^{-1} (50 % CLs: 0.05, 0.07; assuming sex ratio recorded for the site from genetic sampling) for the area of the entire acoustic array. At Kiwarrak, a crude density estimate of 0.04 koalas ha^{-1} was calculated for a search area of 208 ha (52 % of array). This was slightly lower than the acoustic estimate of 0.06 koalas ha^{-1} (50 % CLs: 0.03, 0.06; assuming sex ratio recorded for the site from genetic sampling) for the area of the entire acoustic array, but this was within the bounds of error. The mean acoustic-derived density for just the portion of each acoustic array that was searched by dogs did not differ from estimates for the entire array at each site (0.07 and 0.06 for Kalateenee and Kiwarrak, respectively).

Discussion

In agreement with previous studies in other koala populations (Martin 1985; Penn et al. 2000; Watchorn and Whisson 2019), this study demonstrated that koala sex ratios at two study sites on the north coast of NSW did not differ from parity, allowing acoustic assessments of male koala density to be extended to whole population estimates at these sites. Additionally, this study provided crude estimates of koala density using scat sampling in association with genotyping. Estimates were broadly consistent with those obtained from acoustic surveys and spatial count modelling at the sites, providing cross validation of the acoustic method (see also Law et al. in press for additional validation). The genetic data also revealed spatial genetic structuring of male koalas at one of these sites, providing insights into the spatial clustering of kin and potential dispersal patterns.

One of the limitations of acoustics for estimating koala density is that an assumption needs to be made about the sex ratio of the population of study since it is primarily male koalas that are detected. Estimates in the literature indicate koala populations typically occur in a 1:1 sex ratio (Martin 1985; Penn et al. 2000; Watchorn and Whisson 2019). In our study areas, koala sex ratios did not differ from parity, though sample sizes were relatively small. This was particularly the case for Kiwarrak where the success rate of sex-typing was ~50 % lower than Kalateenee. It is unclear why there was a difference between sites, but it is likely due to sample quality as DNA concentration obtained from the Kiwarrak samples was lower than that obtained from Kalateenee (unpublished data – M. Blyton & K. Brice). DNA degradation and the loss of surface mucous containing most koala DNA, is greater when biological samples are exposed to rain (Piggott 2004; Wedrowicz et al. 2013). In the month prior to scat sampling at Kalateenee, ~25 mm of rain was recorded at nearby Kempsey Airport (BOM weather station 59007), whereas for Kiwarrak there was 3-times more rain (~76.4 mm) recorded at nearby Wingham (BOM weather station 060036) potentially exposing some older samples to rain prior to collection. Furthermore, the moderate severity burn at the Kiwarrak study area affected parts of the canopy, reducing shelter from the rain. In the absence of rain, or where scats occur in areas protected from

weather, scat samples up to 4 weeks old should produce DNA quality that can be reliably used for genotyping studies (Wedrowicz et al. 2013).

Crude density estimates obtained from genetic sampling were within the range of acoustic estimates. The number of unique individual koalas detected at multiple points at each site was too small to allow for spatially-explicit-capture-recapture assessment of density, suggesting use of scats and detection dogs for more precise density estimates is likely to be difficult. Nevertheless, differences in estimates between genetic and acoustic approaches were within bounds of uncertainty (calculated for the latter). It is important to recognise that these differences may be influenced by the different scales of assessment. Acoustic sampling covered a large area (~400 ha), including deep gullies, away from roads, whereas genetic assessments using koala scat detection dogs were limited to areas with suitable topography and were unable to efficiently sample sites with post-fire regeneration of dense shrubs. As a result, genetic assessments can miss koalas in some of these inaccessible parts of the study area, particularly if koalas are more likely to use these parts of the landscape, and therefore provide an underestimate of koala density. This may be particularly important in post-fire landscapes where deep gullies can contain unburnt or low severity fire refugia. Nevertheless, there was broad agreement between approaches for estimates of density. Koala density can be broadly classified as “low” density populations (typically <1 individual ha^{-1} (Phillips and Callaghan 2011; Close et al. 2017), “moderate” (1-3 individuals ha^{-1}) and “high” (4-8 individuals ha^{-1}) (Melzer et al. 2000). In the two areas assessed during the current study, estimates derived from acoustic and genetic techniques indicated low density populations at both sites. Similar estimates have been reported for koalas in other forests on the north coast of NSW (Bongil Bongil NP: 0.07 individuals ha^{-1}; Smith 2004 and 0.1 individuals ha^{-1}; Law et al. in press). It should be noted that the estimate for Kiwarrak is one year post- 2019 Hillville mega fire.

Genetic data provided additional population information at one of the study sites (Kalateenee). There was spatial genetic structure among male koalas at this site,

indicating that male koalas within 400 m of each other were more likely to be close relatives than those further apart. However, there was no evidence of this for female koalas. The presence of male genetic structure in the absence of female genetic structure over the same spatial scale could indicate female biased dispersal in this population (Banks and Peakall 2012). Further analysis with larger sample sizes is required to confirm this. Elsewhere, female biased dispersal has been reported for adult koalas, whereas, a higher proportion of young male koalas dispersed relative to young females (Dique et al. 2003). As well as providing information about the genetic structure within koala populations, it may be possible to assess the degree of gene flow between populations, highlighting the additional value that genotyping of koala scats can provide.

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References

Banks, S. C., and R. Peakall. 2012. Genetic spatial autocorrelation can readily detect sex-biased dispersal. *Molecular Ecology* 21:2092-2105.

Bruford, M. W, Hanotte, O., Brookfield, J. K. Y., & Burke, T. (1998). Multilocus and single-locus DNA fingerprinting, p 287-336. *In* Hoelzel AR (ed), Molecular Genetic Analysis of Populations: A Practical Approach. IRL Press, Oxford.

Chandler, R. B., & Royle, J. A. (2013). Spatially explicit models for inference about density in unmarked or partially marked populations. *The Annals of Applied Statistics*, 7: 936-954.

Close, R., Ward, S., & Phalen, D. (2017). A dangerous idea: that Koala densities can be low without the populations being in danger. *Australian Zoologist*, 38: 272-280.

Cristescu, R., Cahill, V., Sherwin, W.B., Handasyde, K., Carlyon, K., Whisson, D., Herbert, C. A., Carlsson, B. L. J., Wilton, A. N., & Cooper, D. W. (2009). Inbreeding and testicular abnormalities in a bottlenecked population of koalas (*Phascolarctos cinereus*). *Wildlife Research*, 36:299-308.

Dennison, S., Frankham, G., Neaves, L., Flanagan, C., FitzGibbon, S., Eldridge, M., & Johnson, R. (2017). Population genetics of the koala (*Phascolarctos cinereus*) in north-eastern New South Wales and south-eastern Queensland. *Australian Journal of Zoology*, 64:402-412.

Dique, D. S., Thompson, J., Preece, H. J., de Villiers, D. L., & Carrick, F. N. (2003). Dispersal patterns in a regional koala population in south-east Queensland. *Wildlife Research*, 30: 281-290.

Houlden, B., England, P., & Sherwin, W. (1996). Paternity exclusion in koalas using hypervariable microsatellites. *Journal of Heredity*, 87:149-152.

Kavanagh, R. P., & Stanton, M. A. (1995). Nocturnal birds and non-flying mammals of the Dorrigo three-year Environmental Impact Statement Area, Northern Region. New South Wales. Forest Resources Series, 26.

Kavanagh, R. P., Debus, S., Tweedie, T., & Webster, R. (1995). Distribution of nocturnal forest birds and mammals in north-eastern New South Wales: relationships with environmental variables and management history. *Wildlife Research*, 22: 359-377.

Law, B. S., Brassil, T., Gonsalves, L., Roe, P., Truskinger, A., & McConville, A. (2018). Passive acoustics and sound recognition provide new insights on status and resilience of an iconic endangered marsupial (koala *Phascolarctos cinereus*) to timber harvesting. *PloS One*, 13: e0205075.

Law, B., Gonsalves, L., Burgar, J., Brassil, T., Kerr, I., Wilmott, L., Madden, K., Smith, M., Mella, V., Crowther, M., Krockenberger, M., Rus, A., Pietsch, R., Truskinger, A., Eichinski, P., & Roe, P. (in press). Validation of Spatial Count Models to Estimate Koala *Phascolarctos cinereus* Density from Acoustic Arrays. *Wildlife Research*.

Loebel, D. A. F, & Johnston, P. G. (1997). Analysis of the intron-exon structure of the G6PD gene of the wallaroo (*Macropus robustus*) by polymerase chain reaction. *Mammalian genome*, 8:146.

Loebel, D., Longhurst, T., & Johnston, P. (1995). Full-length cDNA sequence of X-linked G6PD of an Australian marsupial, the wallaroo. *Mammalian Genome*, 6:198-201.

Martin, R.W. (1985). Overbrowsing, and decline of a population of the koala, Phascolarctos cinereus, in Victoria. III. Population dynamics. *Wildlife Research*, 12:377-385. doi:10.1071/WR9850377

Melzer, A., Carrick, F., Menkhorst, P., Lunney, D., & John, B. S. (2000). Overview, critical assessment, and conservation implications of koala distribution and abundance. *Conservation Biology*, 14: 619-628.

Peakall, R., & Smouse, P. E. (2006). GenAlEx 6: genetic analysis in Exel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6:288-295.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Exel. Population genetic software for teaching and research - an update. *Bioinformatics*, 28:2537–2539.

Penn, A.M., Sherwin, W.B., Gordon, G., Lunney, D., Melzer, A., & Lacy, R.C. (2000). Demographic forecasting in koala conservation. *Conservation Biology*, 14:629-638.

Phillips, S., & Callaghan, J. (2011). The Spot Assessment Technique: a tool for determining localised levels of habitat use by Koalas *Phascolarctos cinereus*. *Australian Zoologist*, 35: 774-780.

Piggott, M. P. (2004). Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. *Wildlife Research*, 31: 485-493.

Ruiz-Rodriguez, C. T, Ishida, Y., Greenwood, A. D., & Roca, A. L. (2014). Development of 14 microsatellite markers in the Queensland koala (*Phascolarctos cinereus adustus*) using next generation sequencing technology. *Conservation Genetics Resources*, 6:429-431.

Smith, A. P., Andrews, S. P, & Moore, D. M. (1994). Terrestrial Fauna of the Grafton and Casino State Forest Management Areas: Description and assessment of impacts. EIS Report to State Forests NSW, Sydney Australia,1994.

Smith, A. P. (2004). Koala conservation and habitat requirements in a timber production forest in north-east New South Wales. *Conservation of Australia's Forest Fauna,* 2: 591-611.

Smouse, P. E., & Peakall, R. (1999). Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity,* 82:561-573.

Watchorn, D.J., and Whisson, D.A. (2019). Quantifying the interactions between koalas in a high-density population during the breeding period. *Australian Mammalogy,* 42:28-37. doi:10.1071/AM18027

Wedrowicz, F., Karsa, M., Mosse, J., & Hogan, F. E. (2013). Reliable genotyping of the koala (*Phascolarctos cinereus*) using DNA isolated from a single faecal pellet. *Molecular Ecology Resources,* 13: 634-641.

Wedrowicz, F., Mosse, J., Wright, W., & Hogan, F. E. (2018). Genetic structure and diversity of the koala population in South Gippsland, Victoria: a remnant population of high conservation significance. *Conservation Genetics,* 19: 713-728.