Piping call diversity in the crimson rosella species complex: evidence of individual signalling

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Statement of Responsibility

This Thesis is submitted in accordance with the regulations of Deakin University in partial fulfillment of the requirements of the degree of Bachelor of Science Honours. I, Erika Roper, hereby certify that the information presented in this thesis is the result of my own research, except where otherwise acknowledged or referenced, and that none of the material has been presented for any degree at another university or institution.

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This project DID NOT involve the use of animal or human subjects requiring Deakin University ethics committee clearance, nor a permit from any external organisation.

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Abstract

Birds communicate using a wide variety of signals, including auditory, visual, and olfactory signals. Avian vocalisations have various functions, ranging from mate attraction, mate choice, and territorial defence, to the recognition of kin, species, or individuals. In order to perform these functions, vocalisations must vary, and often provide honest indicators of individual condition or quality, or individual identity. This study aimed to test for variation in the piping call of the crimson rosella, *Platycercus elegans*, species complex, in order to determine the possible function of the piping call. I analysed 480 calls from 89 unmarked individuals representing three subspecies of the crimson rosella, and a hybrid population. Ten acoustic variables were tested (call duration, number of elements, element duration, inter-element silence duration, risetime, falltime, maximum frequency, minimum frequency, peak frequency, and frequency bandwidth). This study shows that crimson rosella piping calls have high levels of individual variation, especially in frequency variables. I propose that local populations may have consistent basic call structure (call duration, number of elements), but that individual rosellas within populations could use distinctive frequency combinations as individual signals. There was little evidence to show that crimson rosellas display subspecies or ontogenetic variation in their piping calls. The findings of this study could be further tested with the use of playback experiments, and the study of piping calls of marked birds.

Acknowledgements

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Introduction

Phenotypic variation is one of the main underlying drivers of speciation, as naturally and sexually selected traits are often implicated in species recognition (Price, 1998). Examples of phenotypic variation include variations in morphological characteristics of an animal, or variation in vocalisations. Vocalisations fulfil a range of functions in many animals, such as mate choice, mediating social interactions, and signalling qualities of individuals, such as sex, age, or identity (Bradbury and Vehrencamp, 2011). Studies that examine patterns of variation in vocalisations within and between populations can give insights into these functions.

1.1 Acoustic communication in birds

Birds communicate using a wide variety of signals, including auditory, visual, and olfactory signals (Bradbury and Vehrencamp, 2011). Bird vocalisations have various functions, ranging from territorial defence, mate attraction, and mate choice, to the recognition of species, kin, or individuals (Catchpole and Slater, 2008, Marler and Slabbekoorn, 2004). In order for vocalisations to perform these functions, they must vary, and they often provide honest indicators of individual condition or quality, or individual identity (Marler and Slabbekoorn, 2004).

Vocal learning is the ability of an animal to learn new vocalisations over the course of its life, as opposed to learning all of its vocalisations during a brief period of early life (Marler and Slabbekoorn, 2004). Vocal learning has arisen in three avian taxa, the oscine songbirds (Passeriformes, e.g. Irwin, 2000, Koetz et al., 2007), hummingbirds (Apodiformes, e.g. Gaunt et al., 1994), and parrots (Psittaciformes, e.g. Bond and Diamond, 2004, Kleeman and Gilardi, 2005, Wright, 1996). Marler and Slabbekoorn (2004) propose that vocal variation, variation in the vocalisations of different groups, (e.g. populations, age groups, or individuals) is limited to vocal learners.

Vocal variation may allow for the recognition of subspecies or natal groups, and also enables recognition of familiar individuals in many species, such as parent-offspring relationships (e.g. Pitcher et al., 2012), family relationships (e.g. Janik et al., 1994, Wanker et al., 1998, Wanker et al., 2005), and neighbour or stranger relationships (e.g. Dahlin and Wright, 2012, Wright and Dorin, 2001).

1.2 Variation in parrot vocalisations

Parrots are vocal learners, and retain the ability to learn new vocal elements over the course of their lives, as shown by Pepperberg in her iconic studies on parrot learning and cognition (Pepperberg, 2006, Pepperberg, 2010). Vocal learning may be especially beneficial to parrots as they live in fission-fusion societies, social groups that are in a constant state of flux (Bradbury and Vehrencamp, 2011). Population or individual vocal signatures may be beneficial for parrots to enable them to differentiate between different groups or individuals on a local scale. As individuals move from place to place during the day, having the ability to recognise one's own group (roost, feeding flock) or mate at a distance would be greatly advantageous, enabling avoidance of potentially disastrous attempts to join the wrong group (Andrew, 1962). Some parrot species have been shown to modify their vocalisations over the course of a single new interaction (Scarl and Bradbury, 2009), and others appear to have the ability to learn local dialects upon immigrating into a new populations after dispersal (Wright et al., 2005). Despite their complex vocalisations and remarkable vocal learning abilities, the vocalisations of parrots have been studied relatively little compared to other avian taxa and in most species their functions are unknown.

1.2.1 Geographic variation in parrot vocalisations

Geographic variation in parrot vocalisations tends to occur in one of two patterns, either along a gradient, or as distinct regions with little geographical overlap of dialects (Bradbury et al., 2001). Examples of graded variation include contact calls of the kea (*Nestor notabilis*, Bond and Diamond, 2004), and the orange-fronted conure (*Aratinga canicularis*, Bradbury et al., 2001). Examples of distinct regional dialects include the contact calls of the yellow-naped amazon (Wright, 1996, Wright and Dorin, 2001), crimson rosella (*Platycercus elegans*, Ribot et al., 2009), Australian ringneck (*Barnardius zonarius*, Baker, 2011, Baker, 2000, Baker, 2008) and feral monk parakeets (*Myiopsitta monachus,* Buhrman-Deever et al., 2007)

For example, (Wright, 1996) showed distinct dialects in the contact calls of yellow-naped amazons in Costa Rica, and found that not only did the sampled populations fall into three broad dialect regions, there was also fine-scale variation among the different populations within a dialect. Playback studies on yellow-naped amazons (Wright and Dorin, 2001) and orange-fronted conures (Vehrencamp et al., 2003) found differences in the response of the birds to calls from local (same dialect) and non-local populations (different dialect), finding a greater response by birds to local calls than non-local calls. These findings indicate that parrots are able to gauge the familiarity of individuals from different populations based on their calls.

1.2.2 Individual variation in parrot vocalisations

Individual variation is defined as "when an organism identifies another individual according to its distinctive characteristics" (Dale et al., 2001) and has been demonstrated in visual signals (Dale et al., 2001), olfactory signals (Hagelin and Jones, 2007), and auditory signals (Clark et al., 2006). Individual variation is key to individual recognition, and has been documented in many species (reviewed by Tibbetts and Dale, 2007). Tibbetts and Dale (2007) propose that acoustic signals used in the identity of individuals will occur when confusion between individuals is likely to be costly, and that territoriality, dominance hierarchies, complex social interactions and colonial breeding may favour identity signalling. Several studies have focused on call diversity at an individual level (Cortopassi and Bradbury, 2006, Wanker et al., 1998, Wanker et al., 2005) and recognition of individual vocal signals has been documented in many species (Clark et al., 2006).

Dale et al. (2001) developed six properties that signals that function for individual recognition are expected to exhibit. Such signals should show: 1) negative frequency-dependent selection (rare morphs are at a selective advantage); 2) complex, multimodal frequency distributions; 3) should be relatively cheap and not condition-dependent; 4) different signal variants have equal fitness at equilibrium; 5) an independent assortment of component characters; and 6) occur as fixed phenotypes with high degrees of genetic determination.

Individual vocal variation and the ability to signal and recognise individuals and groups is important to social species such as parrots, especially those with long lasting pair-bonds and relatively long periods of young dependence (Saunders, 1983, Wooller et al., 1983). Several studies have shown that parrots are able to distinguish different individuals by call only, indicating that the calls involved must be specific to individuals, and may contain individual-specific markers.

Saunders (1983) observed that visually isolated female Carnaby's black cockatoos (*Calyptorhynchus funereus latirostris*) were able to distinguish their mate by their *wy-lah* call, and that visually isolated nestlings were able to recognise the *wy-lah* calls of their parents and begin begging before the parent entered the nest hollow. Saunders (1983) also observed that individuals would call out before joining a flock, and that their mate would leave the flock and join them.

In a series of studies of the vocalisations of spectacled parrotlets, Wanker et al. (1998) have shown that captive individuals are able to distinguish between different social companions by use of a short contact call. Furthermore, they show that certain acoustic parameters may allow spectacled parrotlets to discriminate between individuals and social groups (Wanker and Fischer, 2001), which in turn may define vocal labels that each bird uses to refer to itself, and its conspecifics (Wanker et al., 2005). This indicates that parrots are capable of using vocal signals as individual and social recognition tools instead of relying on visual signals alone.

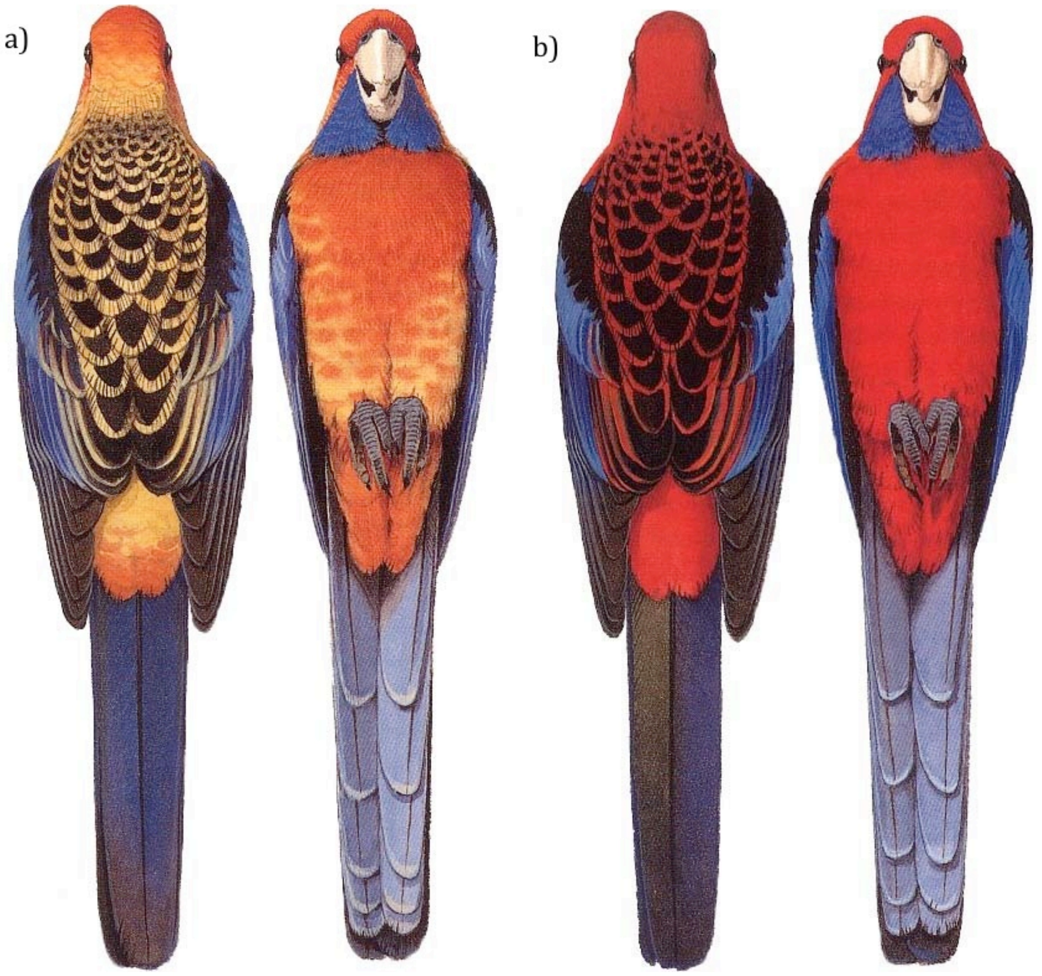
1.2.3 Ontogenetic variation in parrot vocalisations

Ontogenetic variation refers to phenotypic differences that arise at different stages in the life history of a species, and can arise in two forms, variation in the functionally similar calls, and specific call types for different age groups (e.g. nestling begging calls) (Bond and Diamond, 2004). Ontogenetic variation has been investigated in several parrot species to date, though evidence is limited (Bond and Diamond, 2004, Wanker and Fisher, 2001, Berg et al, 2013).

1.3 Study species: the Crimson Rosella

The Crimson Rosella (*Platycercus elegans*) is an abundant species which is commonly classified into seven subspecies defined by plumage colouration and geographic location that are collectively referred to as the crimson rosella species complex. There are 3 main subspecies: 'Crimson Rosellas' (CR, *P. e. elegans*), 'Yellow Rosellas' (YR, *P. e. flaveolus*), and the intermediate forms of the 'Adelaide Rosella' (AR, *P. e. fleurieuensis*, *P. e. adelaidae*, *P. e. subadelaidae*) (Forshaw, 2002, Higgins, 1999). Where the terminal forms CR and YR meet on the western slopes of the Great Dividing Range in southeastern NSW, they hybridise to produce a population of intermediate birds (WS, *P. e. elegans* x *P. e. flaveolus*). The northern QLD and Kangaroo Island populations have not been included (Fig. 1, Fig. 2) in most recent studies of crimson rosellas as they fall outside the geographic ring and are geographically isolated.

Upwards of 20 different calls have been identified for *P. elegans* (Higgins, 1999), though Forshaw (2002) proposes that most are variations of 5 basic call types: 1) Contact calls, given mainly during flight (Ribot et al., 2009); 2) piping calls (Fig. 3), given mainly while perched; 3) twittering/clucking, given at rest; 4) a metallic *chinka-chink*, which accompanies threat postures; and, 5) alarm calls, which are given when birds spot a potential predator (Forshaw, 2002, Ribot et al., 2011).



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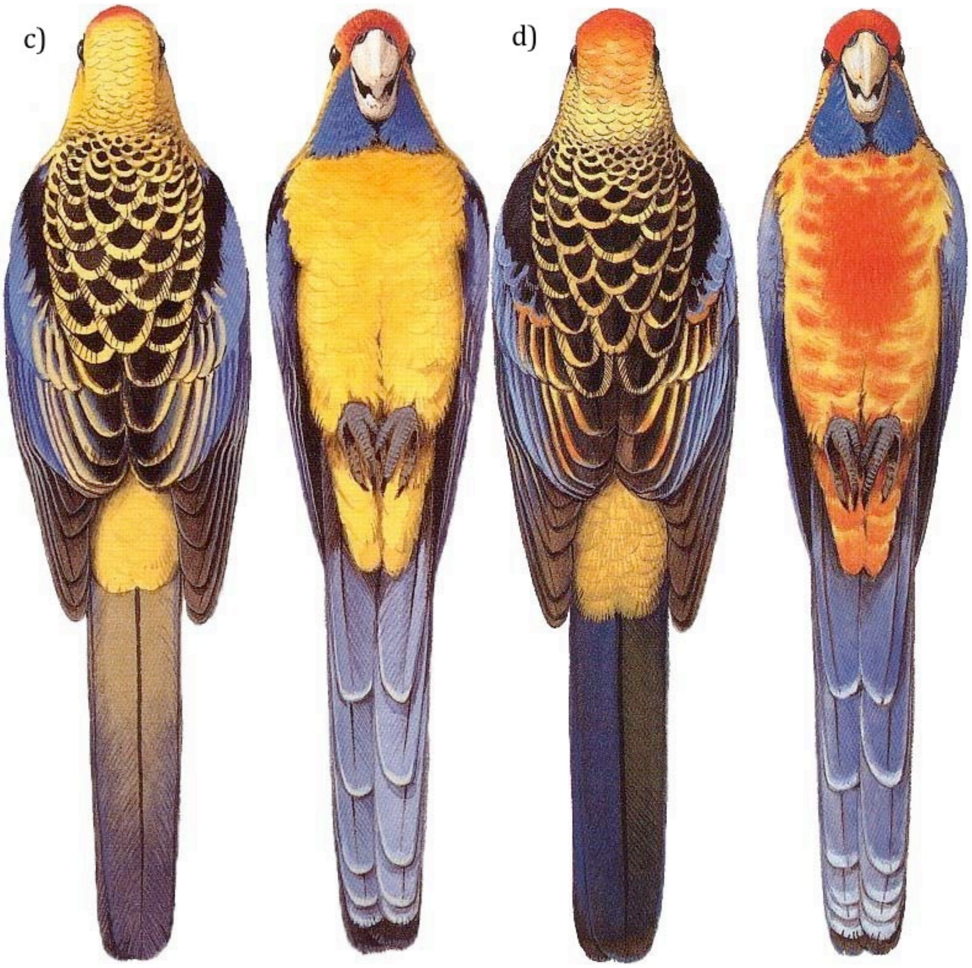


Figure 1. Sample adult plumage colouration of four populations of crimson rosella, *Platycercus elegans*, in southeastern Australia: a) AR, Adelaide rosella, b) CR: crimson rosella, c) YR, yellow rosella, and d) WS: western slopes hybrid. Adapted from Forshaw (2002).

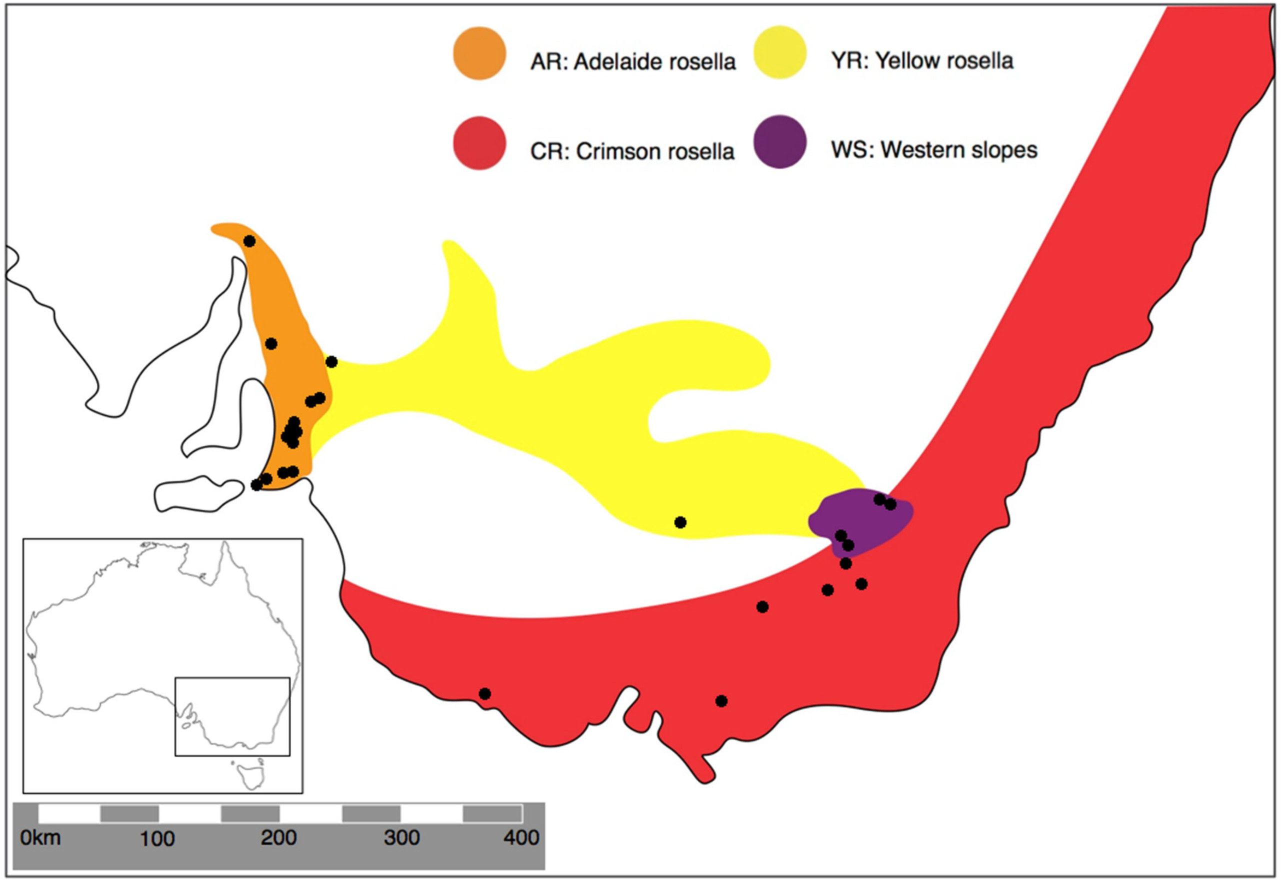


Figure 2. Distribution of *Platycercus elegans* in southeastern Australia, indicating approximate locations of recording sites (closed circles) used for analysis in this study. The colour blocks indicate the distributions of the morphologically distinct Adelaide rosella, crimson rosella, yellow rosella, and western slopes populations where crimson rosella and yellow rosella exist in sympatry (adapted from Forshaw, 2002).

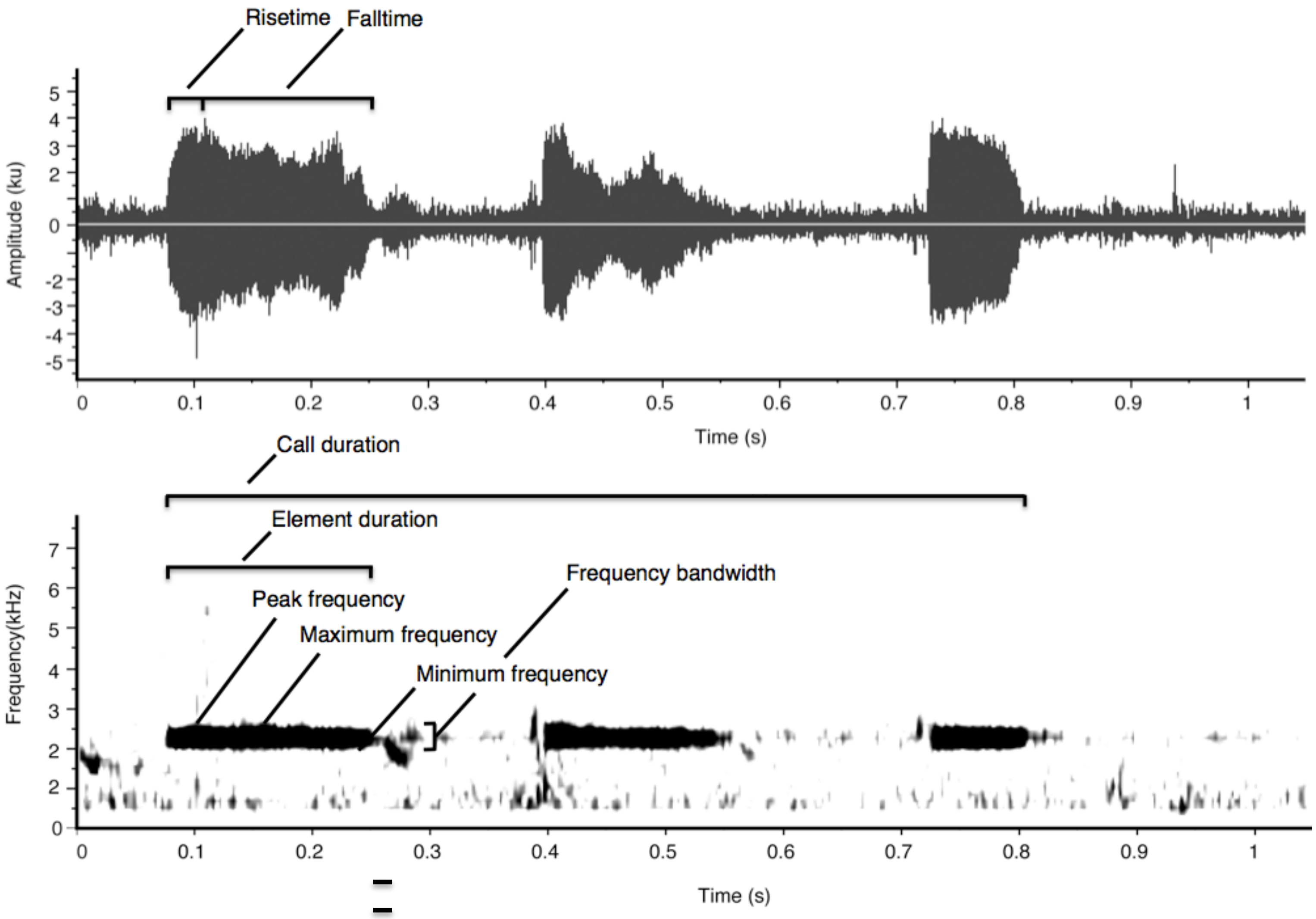


Figure 3. Example (a) waveform, and (b) spectrogram of a three element piping call from an adult Adelaide rosella, *Platycercus elegans adelaidae* (produced in Raven Pro 1.4 and edited in Photoshop CS6).

1.3.1 The crimson rosella contact call

The contact call of the crimson rosella has been studied extensively by Ribot et al. (2012, 2013, 2009). Their aim was to determine whether 1) there was variation in the contact calls between populations, 2) the contact calls of the species may help to maintain differences in plumage colouration between subspecies and 3) whether acoustic variation varied along a gradient that coincided with morphological (plumage colouration) or genetic (microsatellite) changes in that are seen in this species complex.
Geographical differences were observed in the contact call of the crimson rosella, at the level of subspecies, and between local populations (i.e. dialects). Ribot et al. (2009) found that AR and WS had distinctive call types (which were not intermediate between CR and YR), and that CR and YR did not appear to have highly distinctive calls. There was a significant effect of recording site (local population) on all acoustic variables, indicating that local populations may have distinct dialects, and that the contact call may be used in population recognition (Ribot et al 2009). However, playback studies (Ribot et al., 2013) found that individuals of YR, CR, and WS populations responded equally to calls from their own, and other subspecies. This indicates that acoustic variation of the contact call at a subspecies level may not be involved in maintaining the different plumage colours seen between subspecies.

An additional study of the crimson rosella complex found that acoustic variation at the population level in the contact call was associated with a steep microsatellite-inferred genetic cline across geographically continuous populations (YR, WS, and CR) (Ribot et al., 2012). Joseph et al. (2008) found earlier that microsatellite data did not correspond to changes in plumage colouration (YR and WS boundary), but instead occurred about >200km further west, in the YR distribution.

In a study of vocal differences between subspecies in the closely related Australian ringneck parrot, Baker (2011) found that the flight call and beep call of this species (which appear to be equivalent to the contact call and piping call of the crimson rosella respectively (R. Ribot, pers. comm.)) have similar functions of mate contact and recognition. Baker (2011) showed that both the flight call and the beep call followed similar patterns of spatial variation across subspecies. To date, it is not known if the other call types of the crimson rosella show similar patterns of geographic variation as those found in the contact call.

1.3.2 The crimson rosella piping call

Piping calls are multi-syllabic, mellow, whistling calls, which birds often emit while perched (Fig. 3)(Forshaw, 2002, Higgins, 1999). The function of the piping call is currently unknown. It has been suggested that piping calls may be indicators of the location or identity of individuals (Higgins, 1999), however there is no supporting data for this hypothesis.

1.4 Aims

The aims of this study were to

1) test for piping call differences both within and between different subspecies, age groups, and local populations in the crimson rosella *Platycercus elegans* species complex, and
2) determine if the piping call is individually distinctive and may allow for individual signalling and recognition within the crimson rosella species complex.

Specifically, I predicted that in concordance with the findings of Baker (2011) in the Australian ringneck, the piping call should covary along the same geographic pattern as found in the contact call by Ribot et al. (2009).If the piping call serves to maintain phenotypic variation between the subspecies, then the structure of the call would vary significantly between the three phenotypic forms. If the piping call functions for individual identity signalling, I predicted high levels of individual variation in the piping call, as well as evidence for some of the predictors for identity signals put forward by Dale et al. (2001).

Methods

2.1 Vocal recordings: Piping calls

Piping calls were recorded from free-living birds (N = 89) by R. Ribot at 25 sites throughout the range of *P. elegans* in southeastern Australia during the main breeding season (September – January), in daylight hours (Figure 2, Sup. table 1) (Ribot et al., 2009). As soon as possible after an individual was recorded, the next recording would be taken at least 500m away to ensure that the same individual was not recorded multiple times within each site. A global positioning system receiver was used to record the approximate location (±5m) or each recording. At the time of recording, rosellas were observed through binoculars in order to ascertain subspecies and age. Rosellas were identified as subspecies by geographical location and plumage colouration. Birds were then allocated to an age group (juvenile, subadult, or adult) by the percentage of green feathers present on the breast, rump and head as described in Higgins (1999) and Forshaw (2002) (juvenile: < 4 months old, 100% green, subadult: 4 – 12 months old, 10 – 90% green, or adult: > 1 year old, 0% green) (Fig. 4). Full details of recording equipment and collection methods are found in Ribot et al. (2009).

2.2 Acoustic Analyses

Raven Pro version 1.4 (Bioacoustics Research Program, Ithaca, NY, USA 2011) was used to analyse the calls (uncompressed wav format). A bandpass filter (500

– 8000 Hz) was applied to all calls prior to analysis. Additional bandpass filters ranging between 1500 – 3500 Hz were applied on a call-by-call basis to filter out excessive background noise. The following spectrogram settings were used: Blackman window, 400 samples, DFT size: 2048 samples, Overlap: 85, Spectrum range: 18kHz. The Standard Gamma II colour scheme was used to visualise spectrograms as it enabled improved detection and measurement of piping calls as opposed to the default Greyscale setting.

For each call examined, I measured the following acoustic variables which have been shown to be important to parrot communication (Fig.3) (Wanker and Fischer, 2001, Ribot et al., 2009).

Call variables:

1) Total call duration (s)
2) Total number of elements in the call.

Element variables:

3) Element duration (s)
4) Inter-element silence (s), the time between successive elements in a call
5) Risetime (s), the time for the element to reach maximum amplitude
6) Falltime (s), the time from maximum amplitude until the end of the element
7) Minimum frequency (kHz) of each element
8) Maximum frequency (kHz) of each element
9) Peak frequency (kHz) of each element
10) Frequency bandwidth (kHz) of each element.

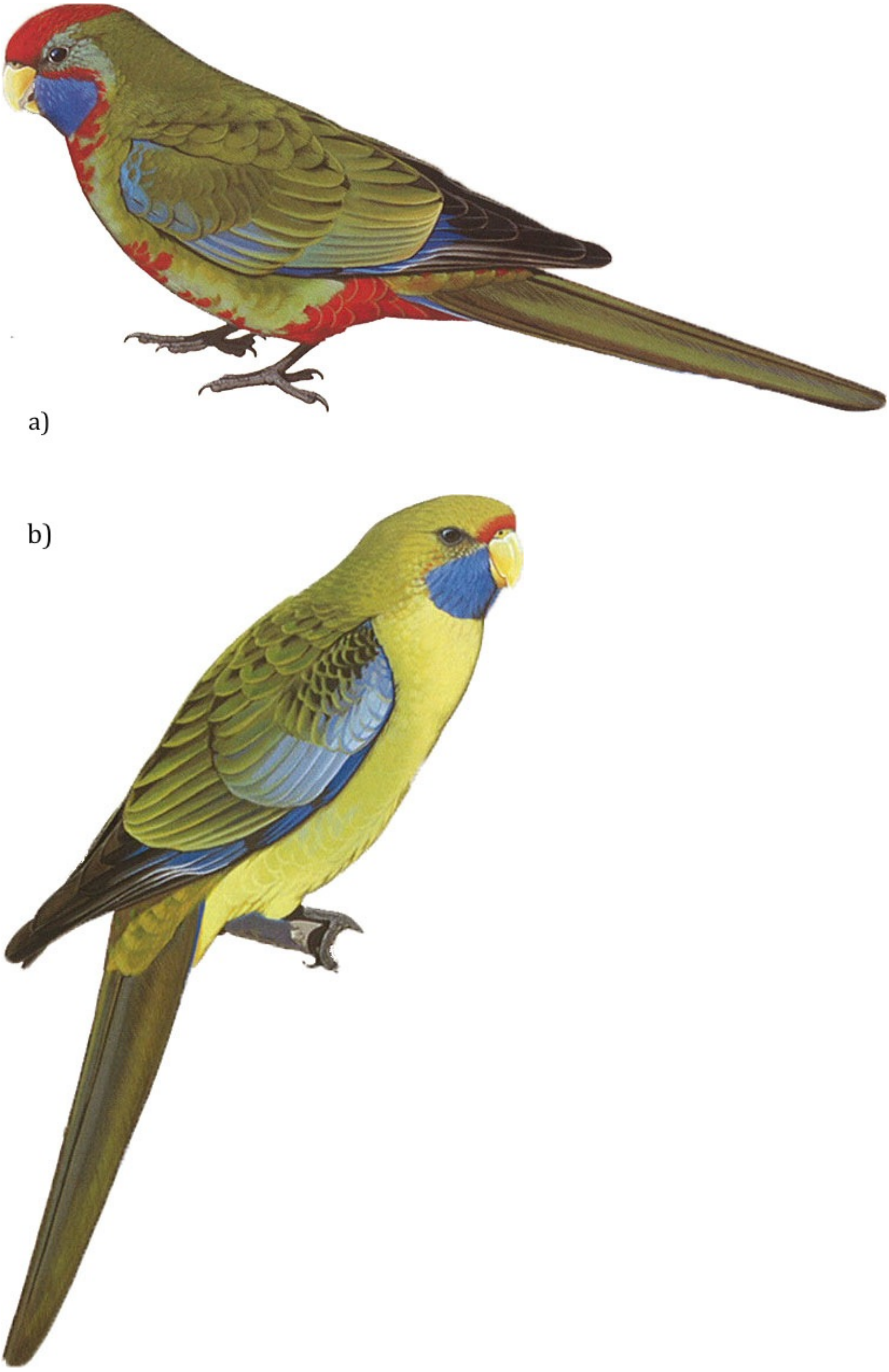


Figure 4. Example plumage colouration of juvenile crimson rosellas: a) CR, crimson rosella, b) YR, yellow rosella. Adapted from Higgins (1999).

All calls of sufficient quality were measured for call variables. For recordings that contained ≤5 calls, all calls of sufficient quality were also analysed for element variables. For recordings that contained >5 calls, 5 calls were randomly selected for analysis of element variables. Temporal measurements (acoustic variables 1, 3, 4, 5, and 6 above) were measured from spectrograms (Fig. X) and waveforms using on-screen cursors. Raven Pro automatically measured maximum, minimum, and peak frequency measures, and I calculated frequency bandwidth as the difference between high frequency and low frequency.

2.3 Statistical Analyses

Statistical analyses were performed in SPSS version 21 (IBM Corp., Armonk, NY, USA), and follow Quinn and Keough (2002) and Tabachnick and Fidell (2007). Depending on the acoustic variable being analysed and the type of analysis (linear mixed models or discriminant function analysis), each individual, call, or call element was regarded as a separate data point during analysis, with random intercepts included in mixed models to account for non-independence among data points (as described below).

I ran linear mixed models (MIXED) with restricted maximum likelihood estimation to test for differences in each of the 10 acoustic variables between subspecies, populations, age groups, recording date and, in the case of element variables, element number within the call. Recording day 1 was set as the beginning of the rosella breeding season (October 1st). I analysed each dependent variable separately in order to reveal the effects of specific acoustic variables. Mixed models tested the effects of individual identity, call identity, and recording site (random intercepts) to control for potential non-independence among observations from these groups, while testing the fixed effects of subspecies, age, recording date, and element number within the call. Site was not included as a random variable in mixed model analyses of acoustic variables falltime and frequency bandwidth, as the model would not converge if site were included. Call was only included as a random effect in analyses of acoustic variables minimum frequency, peak frequency and frequency bandwidth, as the model would not converge if call were included in analyses of the other variables. Outliers were identified by examining histograms of model residuals, but in all cases their removal made no qualitative difference to the results. Intraclass correlations (ICC) were calculated from NULL linear mixed model estimates of coefficient parameters for all random effects (individual, call, and site), with no fixed terms included (Tabachnick and Fidell, 2007)

I used discriminant function (DF) analyses to classify individuals by acoustic variables. Four DF analyses were carried out. When classifying individuals to groups (subspecies, site, age), I calculated the variable means of all calls per individual (single point per individual). When classifying calls to individuals, I calculated the variable means for each call (multiple points per individual), however, individuals with only one call were excluded from these analyses. Variables were considered to be strongly loading on discriminant functions (DF) when they had a loading value of >0.4; smaller loadings are not reported in the results for brevity. Acoustic variable frequencies were checked for normality using plots. All p values are two-tailed, and results were considered significant when $p < 0.05$.

Results

I analysed piping calls from 89 individuals throughout the range of the crimson rosella species complex. I analysed 480 calls for the call variable analyses (MIXED), and 288 calls for the element variable analyses (MIXED). Seventy-one individuals were included in the DFA (see Table 1 for variable means and sample sizes).

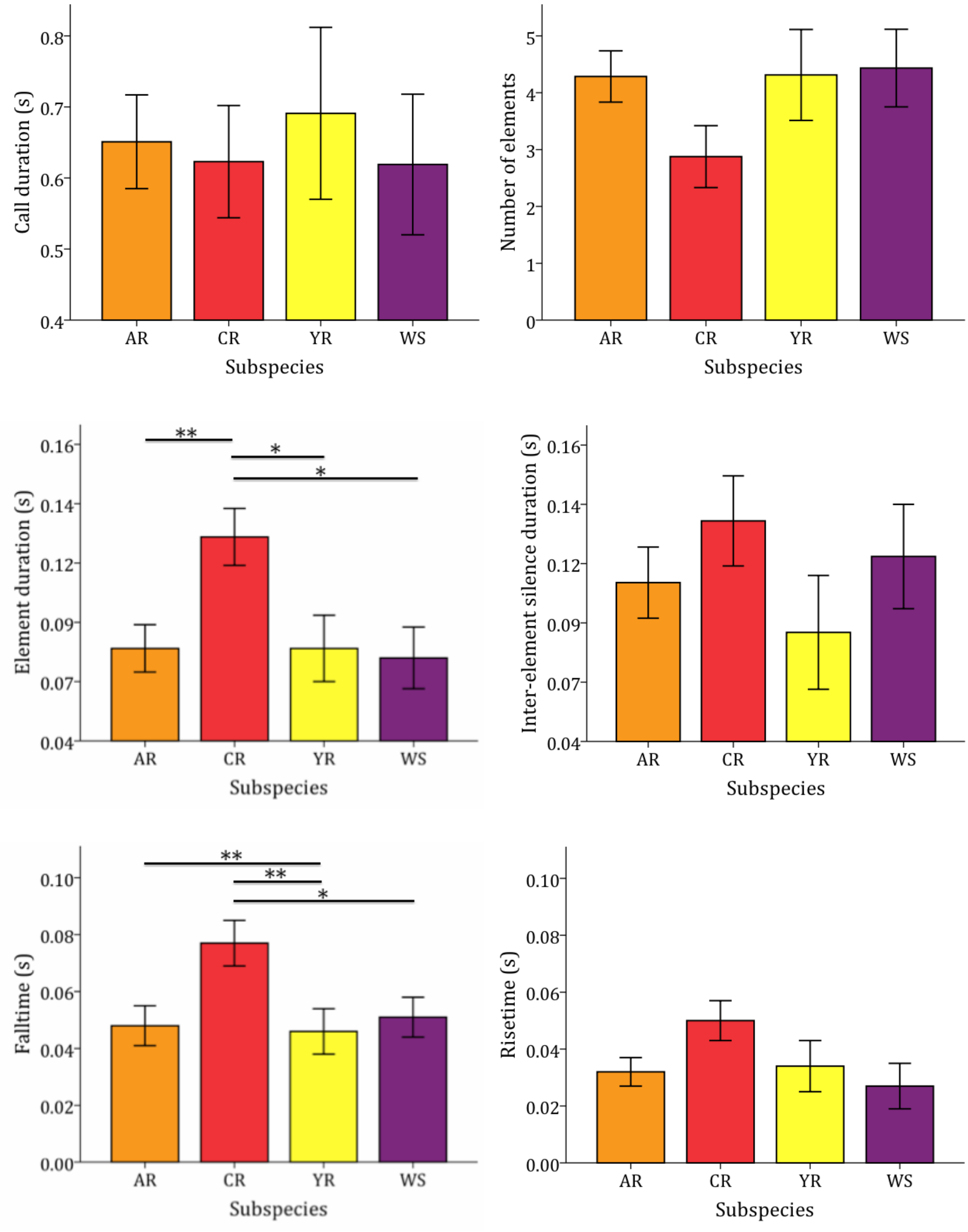
3.1 Subspecies, age and seasonal variation

3.1.1 Subspecies variation

The vocalisations of AR, CR, YR and WS populations differed significantly in the acoustic variable falltime (p = 0.021, CR falltimes were significantly longer), but not in any of the other acoustic variables tested. (Sup. table 2, Fig. 5). However, Fisher's Least Significant Difference (LSD) adjusted post-hoc pairwise comparison tests did reveal that CR had longer element duration than other subspecies (p = 0.007 – 0.029) (Fig. 5).

Table 1. Means, standard errors, ranges, and sample sizes for 10 acoustic variables tested. SD = standard deviation; SE = standard error; N = number of samples.

Variable	Mean	SE	Range	N
Call duration (s)	0.658	0.011	0.070 - 1.51	480 calls
Number of elements	3.910	0.077	1- 10	480 calls
Element duration (s)	0.081	0.001	0.013 – 0.420	1132 elements
Inter-element silence (s)	0.110	0.002	0.007 - 0.383	833 silences
Risetime (s)	0.033	0.001	0.003 - 0.177	1124 elements
Falltime (s)	0.049	0.001	0.005 - 0.359	1123 elements
Minimum frequency (kHz)	2.131	0.010	1.230 - 3.007	1132 elements
Maximum frequency (kHz)	2.565	0.010	1.699 - 3.513	1132 elements
Peak frequency (kHz)	2.360	0.010	1.464 - 3.273	1132 elements
Frequency bandwidth (kHz)	0.434	0.007	0.151 - 1.283	1132 elements



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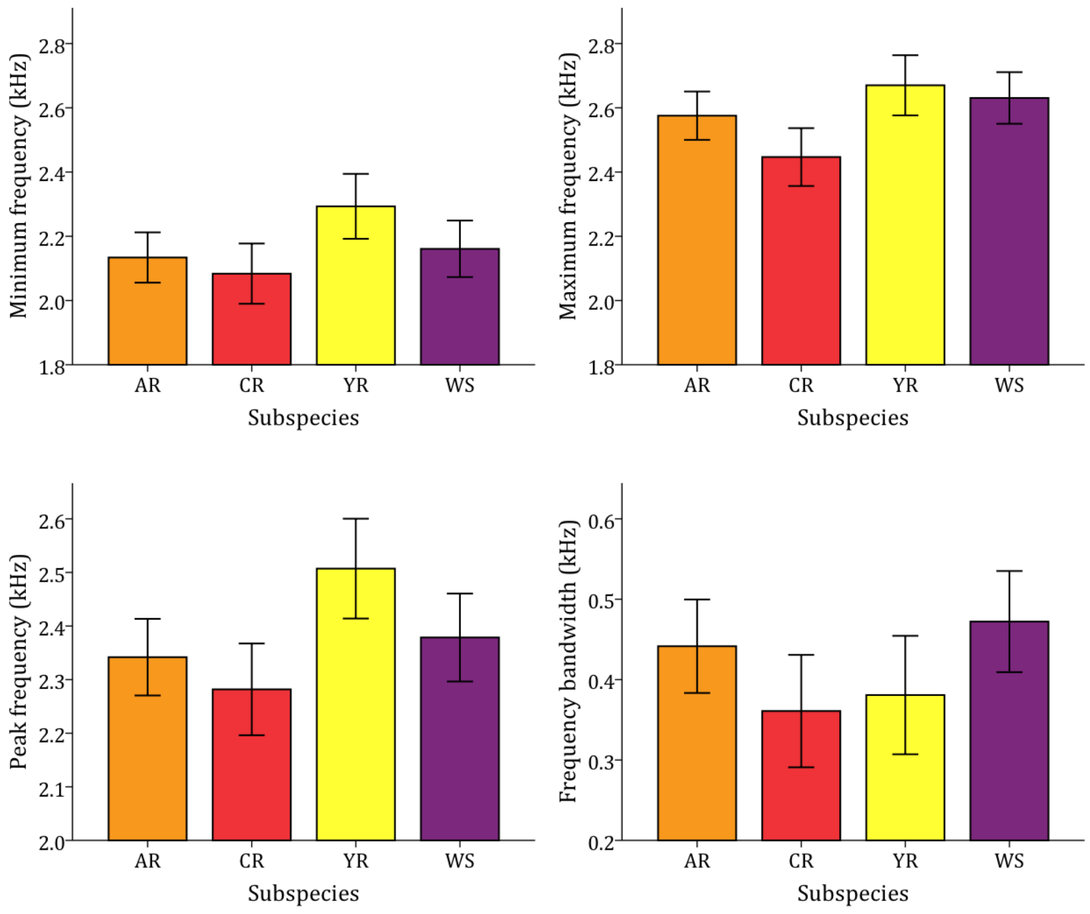


Figure 5. Acoustic variables describing piping calls from four populations of *Platycercus elegans* species complex in southeastern Australia: AR (N = 131), CR (N = 140), YR (N = 62) and WS (N = 147). See text for definition of acoustic variables. Estimated marginal means ± standard error. There are no differences in most acoustic variables. *$p < 0.05$; **$p < 0.005$.

A discriminant function analysis (DFA) was used to classify calls (mean value for each acoustic variable per individual) to groups defined by subspecies (Fig. 6a). This DFA used three discriminant functions (DF). DF1 accounted for 60.9% of the variance in the acoustic variables, and was strongly negatively loaded by element duration (-0.782) and risetime (-0.646), and strongly positively loaded by number of elements (0.524). DF2 accounted for 37.3% of the variance in the acoustic variables, and was strongly negatively loaded by call duration (-0.598). DF3 accounted for 1.8% of the variance seen in the acoustic variables, and was strongly negatively loaded by peak frequency (-0.745), minimum frequency (-0.719) and maximum frequency (-0.579). Overall, the DFA correctly classified 67.6% of individuals to their group. There was good classification of AR (90.0% correctly classified, and 3.3% classified to AR, YR and WS) and CR (71% correctly classified, 21.4 classified as AR, and the remainder classified as WS). YR was poorly discriminated from AR (23.1% classified correctly, 69.2% classified as AR, and the remainder classified as CR), as was WS (57.1% correctly classified, 35.7 classified as AR, and the remainder classified as CR). The acoustic variable falltime was excluded from the analysis as it failed to pass the tolerance test.

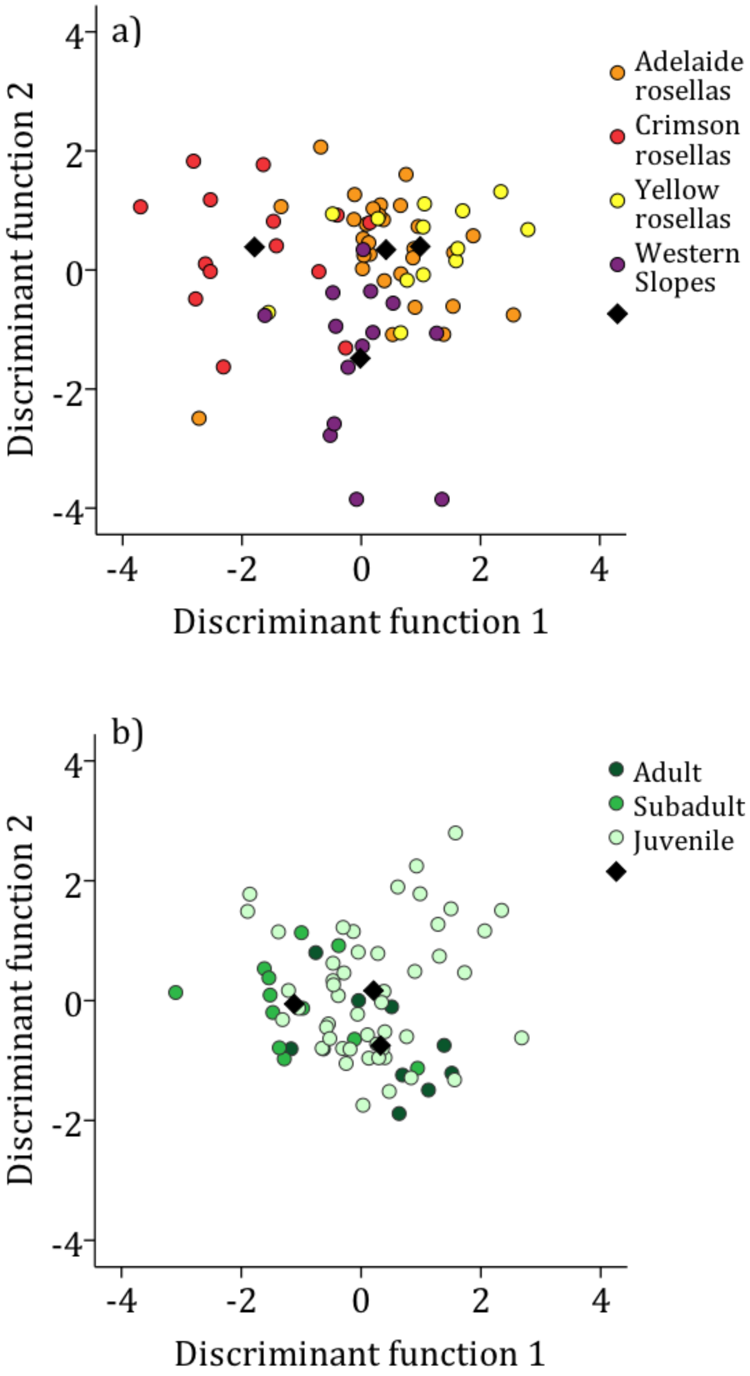


Figure 6. Discriminant function analysis of piping calls based on the ten acoustic variables for a) subspecies (67.6% individuals correctly classified), and b) age group (69.0% individuals correctly classified); each point represents an individual (N=71), 1 – 5 calls per individual, and the diamond symbols indicate centroids.

3.1.2 Ontogenetic variation

The vocalisations of juvenile, subadult and adult birds did not differ significantly in any acoustic variables (Fig. 7, $p > 0.05$).

A second DFA was used to classify calls to groups defined by age (Fig. 6b). This DFA used two DF. DF1 accounted for 72.1% of the variance seen in acoustic variables, and was negatively loaded by call duration (-0.619), and positively loaded by peak frequency (0.584), maximum frequency (0.533) and minimum frequency (0.417. DF2 accounted for 27.9% of variance seen in acoustic variables, and was strongly negatively loaded by minimum frequency (-0.635) and strongly positively loaded by frequency bandwidth (0.462). Overall the DFA correctly classified 69.0% of individuals to their group. There was good classification of individuals to adult (95.9% correctly classified and the remainder classified to subadult). Subadults were poorly discriminated from adults (16.7% correctly classified, and 83.3% classified as adults). Classification of individuals to the juvenile age group was completely unsuccessful (100% classified to adult age group). Acoustic variable falltime was excluded from the analysis as it failed to pass the tolerance test. Overall, subspecies and age were not significant predictors for any of the acoustic variables (Table 3).

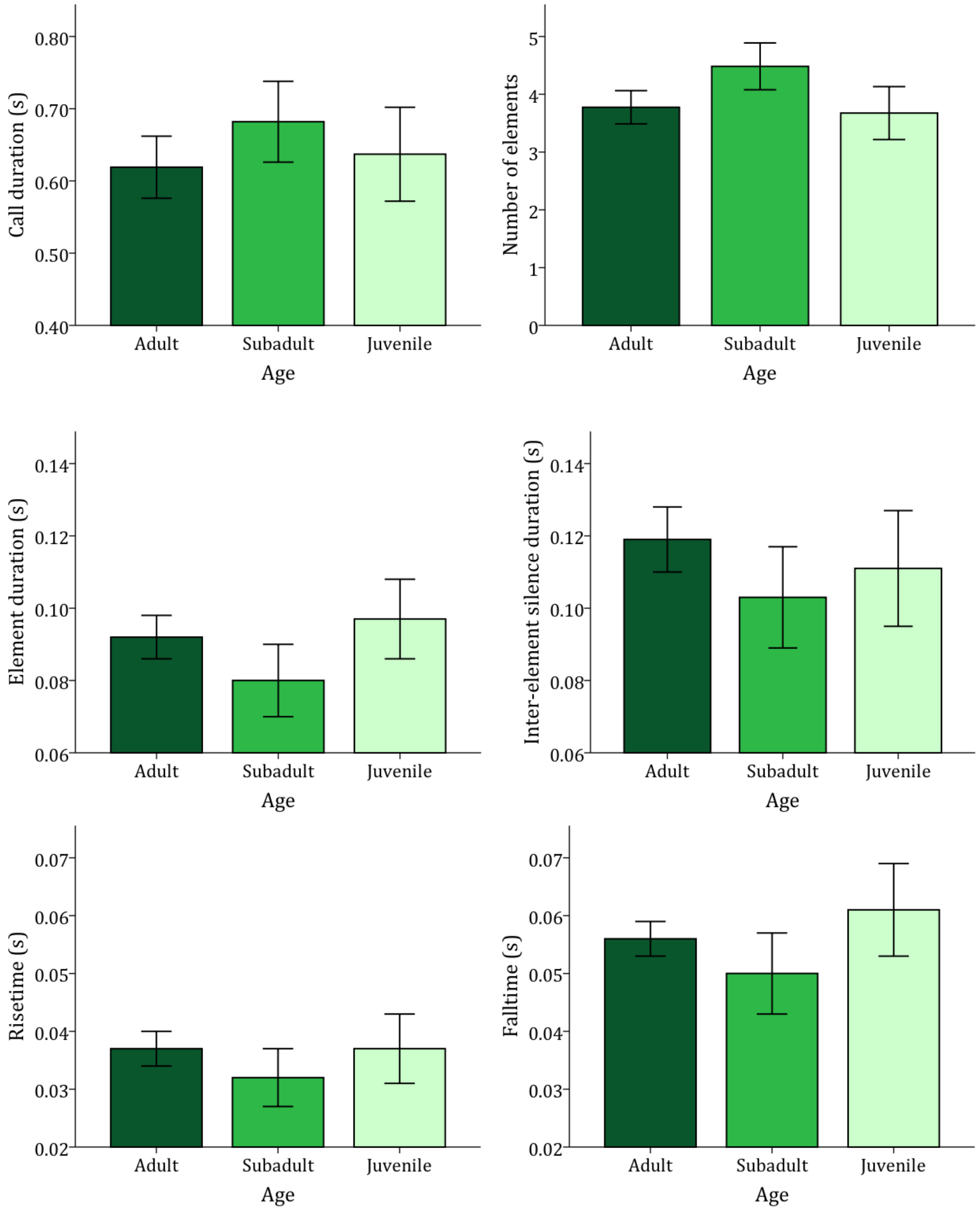
3.1.3 Seasonal variation

I found a significant negative effect of recording date on element duration and falltime. Element number had a significant negative effect on risetime, maximum frequency, peak frequency and frequency bandwidth. However, the magnitudes of these effects were relatively small (Sup. table 2).

3.2 Population and individual variation

3.2.1 Population variation

A third DFA was used to classify calls to groups defined by recording site (considered local populations) (Fig. 8). DF1 accounted for 34% of variance and was positively loaded by risetime (0.507). DF2 accounted for 23.9% of variance, and was strongly negatively loaded by element duration (-0.708), and strongly positively loaded by number of elements (0.481). DF3 accounted for 12.8% of variance, and was strongly positively loaded by call duration (0.732). DF4 – DF9 accounted for the remainder of variance. The solution classified 62.0% of individuals into their correct site overall. Acoustic variable falltime was excluded from the analysis as it failed to pass the tolerance test.



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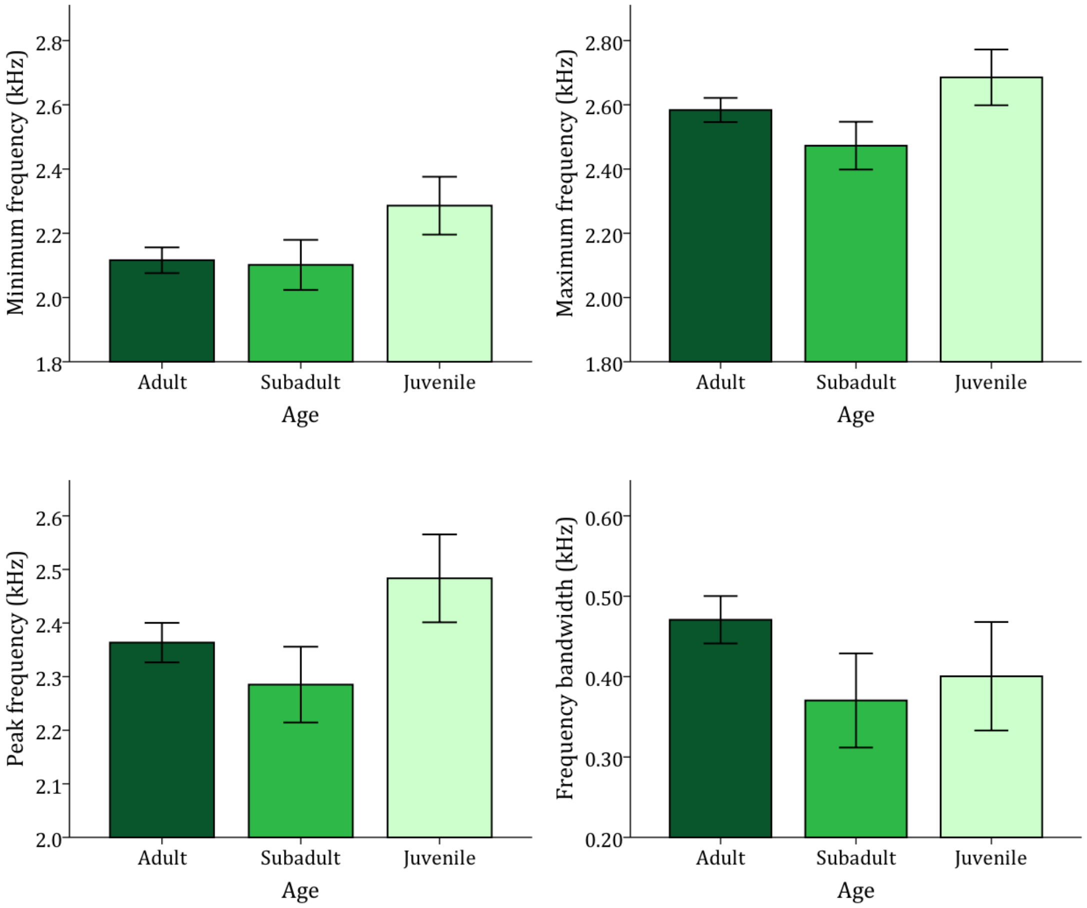


Figure 7. Acoustic variables describing piping calls from three age groups of the *Platycercus elegans* species complex in southeastern Australia: adult (N = 332), subadult (N = 108) and juvenile (N = 40). See text for definition of acoustic variables. Estimated marginal means ± standard error. There are no differences in any acoustic variable

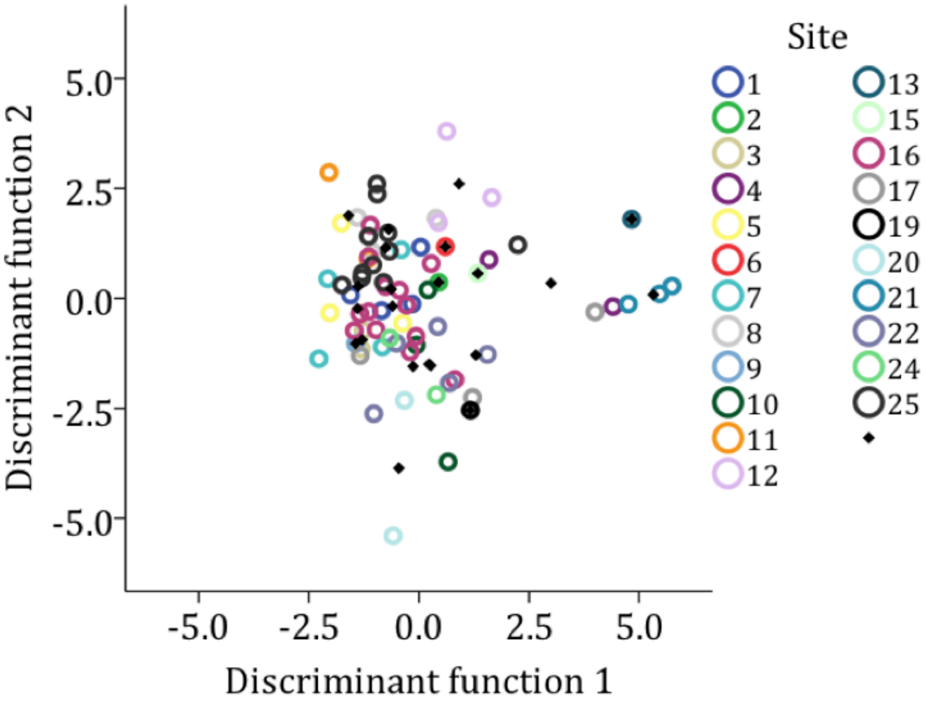


Figure 8. Discriminant function analysis of piping calls based on the ten acoustic variables; each point represents an individual, 1 – 5 calls per individual, and the diamond symbols indicate centroids for the 25 sites (62.0% individuals correcly classified).

3.2.2 Individual variation

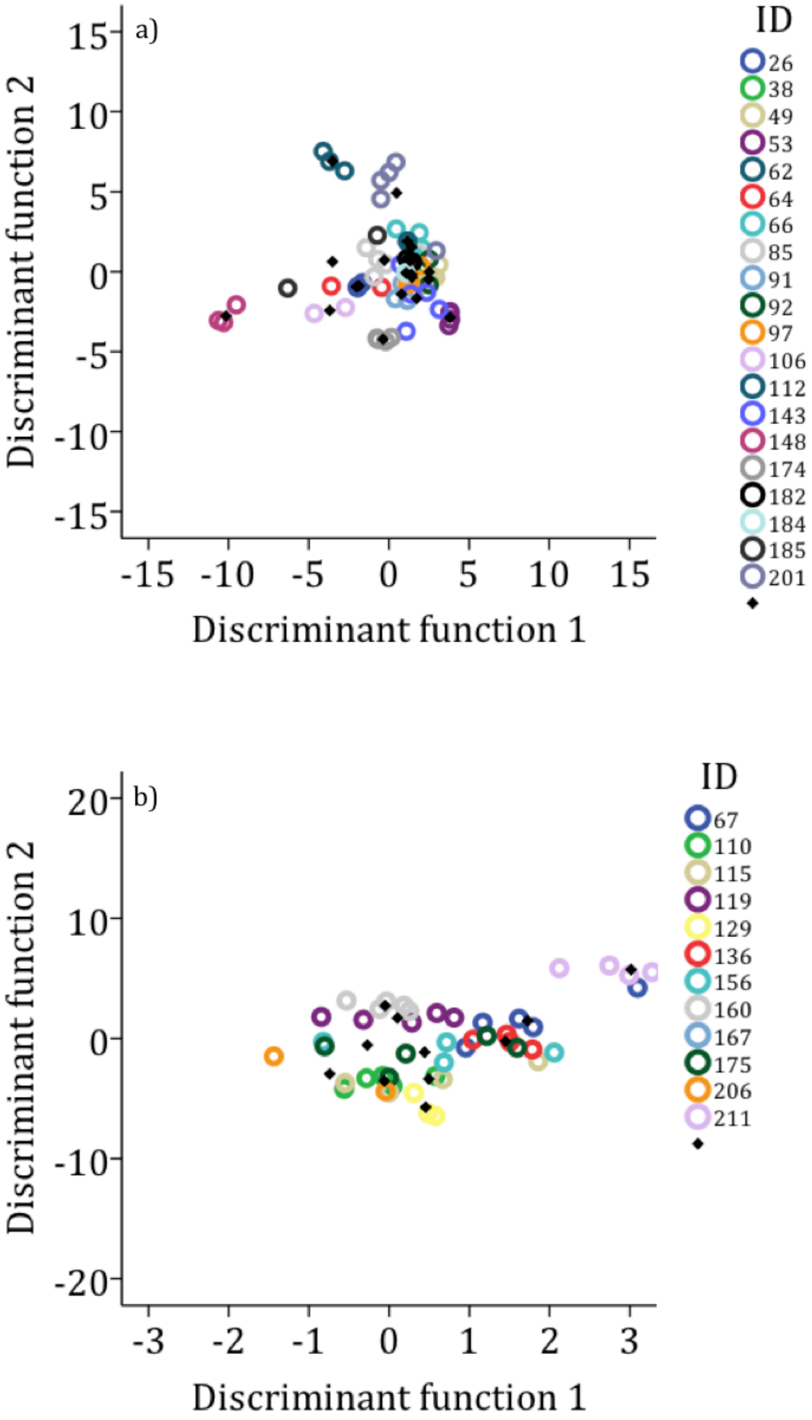
A fourth DFA was used to classify calls to groups defined by individual (not shown). DF1 accounted for 35.9% of the variance seen in acoustic variables, and was negatively loaded by element duration (-0.72) and falltime (-0.601). DF2 accounted for 17.5% of the variance seen in acoustic variables, and was positively loaded by frequency bandwidth (0.662). DF3 accounted for 12.3% of the variance seen in acoustic variables, and was positively loaded by number of elements (0.534) and peak frequency (0.479). DF4 accounted for 11.0% of the variance seen in acoustic variables, and was negatively loaded by peak frequency (-0.684), minimum frequency (-0.664), and maximum frequency (-0.619). DF5 – 10 accounted for the remaining variance seen in acoustic variables. Overall, 67.8% of calls were classified correctly to their group (individual).

Additional DFAs were used to classify the calls of individuals to groups defined by their subspecies or age group (for example, only calls of AR were classified, or only calls of juveniles) (Fig. 9). This led to an increase in correct classification of calls to groups defined by subspecies (AR (90.0% of calls classified correctly), CR (87.7% of calls classified correctly), YR (81.3% of calls classified correctly), and WS (81.5% calls classified correctly)), and to groups defined by age (juvenile (97.0% of calls classified correctly), subadult (83.0% of calls correctly classified), and adult (77.0% calls correctly classified)). When juveniles and subadults were excluded from the analyses (4 – 6 individuals per subspecies), and adult calls were classified within subspecies, correct classification increased further for YR

(100% correctly classified), and WS (95.0% correctly classified), while AR (92.5% correctly classified) and CR (88.0% correctly classified) remained essentially unchanged.

Intraclass correlation coefficients (ICC) indicated that approximately 34 – 44% of the variability in basic piping call structure (call duration and number of elements) is associated with differences between sites (local populations) (Fig. 10). Approximately 18 – 67% of variability in the piping call (all acoustic variables) is associated with differences between individuals. Approx. 56 – 67% of variability in frequency variables (maximum frequency, minimum frequency, peak frequency, and frequency bandwidth) is associated with differences between individuals. Approx. 51 – 57% of variability in inter-element silence duration, risetime, and falltime is associated with within-individual variation (residual).

The ICC (Fig. 10) for individual identity was higher for individual than for site except for variables call duration and number of elements ($p < 0.05$). Significance of the random effect of individual was high for all variables ($p < 0.005$) (see Sup. table 2, Fig. 10).



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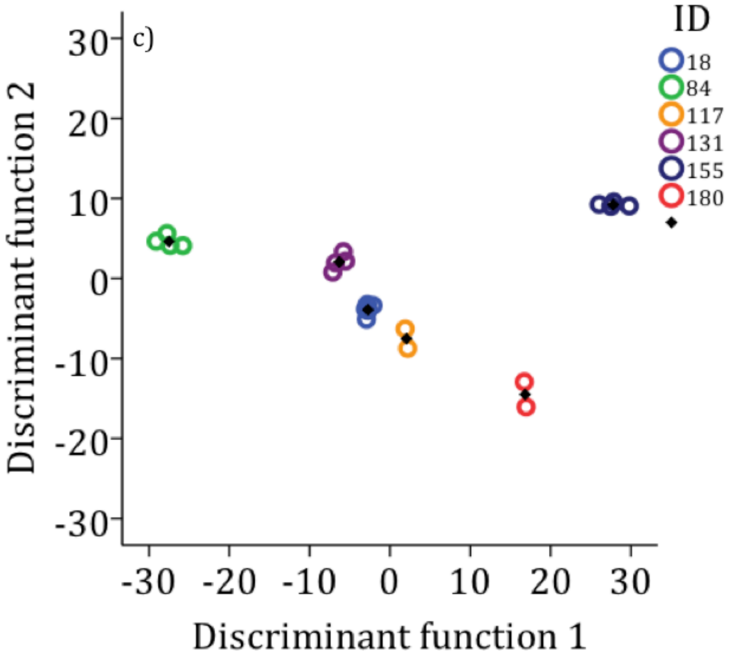

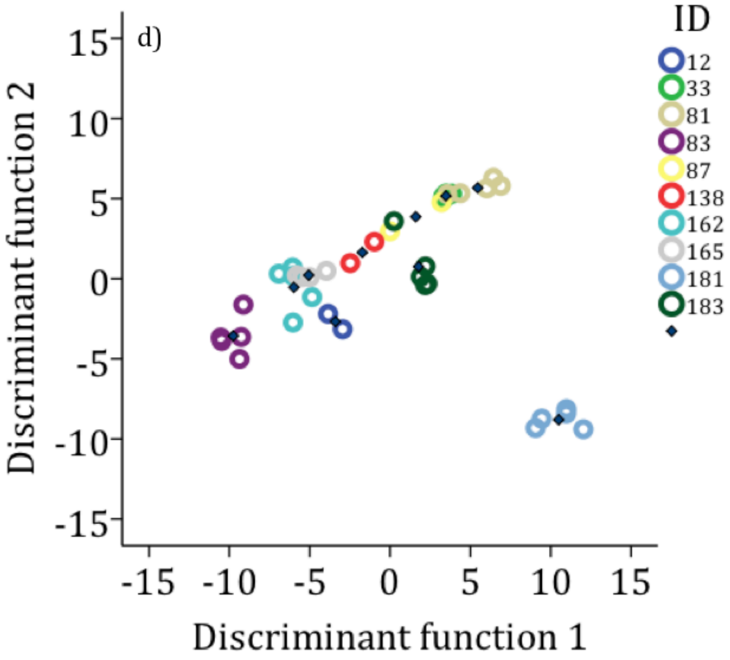

Figure 9. Discriminant function analysis of piping calls based on the ten acoustic variables; each point represents one call, and diamond symbols indicate centroids for the individuals. a) adult AR, b) adult CR, c) adult YR, and d) adult WS.

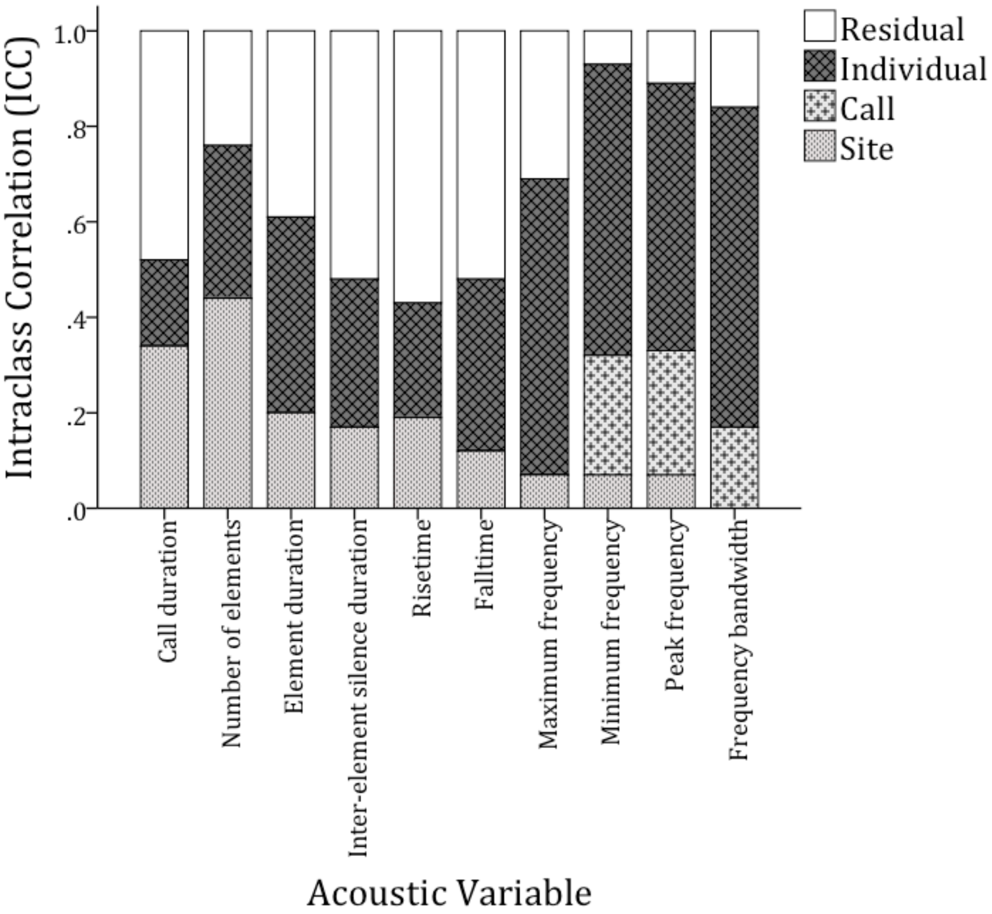

Figure 10. Intraclass correlations for all acoustic variables. Random effects measured are 1) residual effects 2) individual identity of rosella, 3) individual calls, and 4) recording site. Significance of the random effect of individual was high for all acoustic variables ($p < 0.005$). Random effect of site was significant for acoustic variables call duration ($p = 0.035$), number of elements ($p = 0.015$) and risetime ($p = 0.036$), however in all cases site was less significant than individual.

Discussion

4.1 Variation in the piping call

In this study of the piping call of the crimson rosella species complex I analysed ten different acoustic variables in order to test for subspecies, population, age, and individual variation in calls. I found no evidence that the piping call displays geographic variation at the level of subspecies or local population, nor did I find evidence for ontogenetic variation. However, I did find compelling evidence that the piping call exhibits high levels of individual variation, and suggests it could be used by rosellas as an individual signalling and recognition tool.

4.1.1 Individual variation

I found evidence to support the hypothesis that the piping call of the crimson rosella may be used for individual signalling and recognition. Intraclass correlations for individual were consistently high for most acoustic variables, and generally low for recording site (considered a proxy for local population) (Fig. 10). The variability in basic call structure (call duration and number of elements) was mostly explained by recording site, and individual explained the majority of variability found in frequency variables. This suggests that local populations may have consistent basic call structure, but that individual birds within local populations could use distinctive frequency combinations. This is consistent with the findings of Wanker and Fischer (2001), who found high inter-individual variation in frequency parameters (minimum frequency, maximum frequency, peak frequency, and frequency bandwidth) in the contact calls of spectacled parrotlets. Additionally, discriminant function analyses (DFA) revealed that adult rosellas showed consistent individuality in their piping calls (Fig. 9), which is suggested as evidence for individual signals (Wooller et al., 1983).

4.1.2 Geographic and ontogenetic variation

Contrary to the findings of (Ribot et al., 2009), I found little evidence for variation by subspecies in the piping call of the crimson rosella. Analysis of the piping calls of AR, CR, YR, and WS indicates no significant differences between these subspecies in eight out of ten variables: call duration, number of elements, inter-element silence, risetime, maximum frequency, minimum frequency, peak frequency, and frequency bandwidth (Fig. 5). Of these four populations, only 67.6% of individuals were correctly classified to subspecies using a DFA (Fig. 6a). There was a strong tendency to incorrectly classify individuals as AR. Only AR and CR were successfully classified (90% correctly classified), with poor discrimination of YR and WS. CR appears to have longer element duration, which is partly accounted for by longer falltimes, however I suspect these values are unlikely to be biologically significant.

All subspecies could be discriminated with more than 50% accuracy on the basis of my acoustic variables, however accuracy improved when subspecies were analysed separately. These classification values are relatively low when compared to classification of individuals within subspecies.

There was no evidence for variation by recording site in the piping call of the crimson rosella. Analysis of piping calls recorded in sites 1 – 25 indicates no significant difference between the sites in seven variables: element duration, inter-element silence, falltime, maximum frequency, minimum frequency, peak frequency, and frequency bandwidth. However, there was a significant difference between sites for acoustic variables call duration, element number, and risetime (Sup. table 2). Only 62% of individuals were correctly classified to site (Fig. 8). The lack of differences in acoustic variables across subspecies and sites suggests that the piping call is not used for subspecies or group recognition by *P. elegans*.

Additionally, I found no evidence of ontogenetic variation in the piping call of the crimson rosella. Analysis of the piping calls of juveniles, subadults, and adults indicates no significant differences between these age groups in all ten variables tested (Fig. 7). Of these three age groups, only 69% of individuals could be correctly classified to age group using a DFA. No individuals were classified to juvenile, and the majority of individuals were classified to adult. Only adults were correctly classified (95.9%) (Fig.6b).

4.2 Vocal variation in parrots

Vocal variation in the calls of wild parrots has been found in many species to date, in the form of geographic variation, population variation, ontogenetic variation, and individual variation. Individual recognition appears to be used primarily for mate recognition, and parent-offspring recognition (Marler and Slabbekoorn, 2004, Tibbetts and Dale, 2007).

My main findings were consistent with several of the predictions made by Dale et al. (2001) for signals that function for individual recognition rather than quality signalling. The piping call of the crimson rosella is highly variable at an individual level, and does not appear to be condition-dependent. However, as the condition of recorded individuals is unknown, this may not be the case. The piping call does not appear to be environmentally-determined either, as there is no difference in piping calls at a subspecies or population level, which corresponds to different habitats and environmental conditions. The acoustic parameters of the piping call appear to display unimodal frequency distributions (Sup. fig. 2), which is not consistent with the predictions of Dale et al. (2001), however, acoustic variables were tested separately, and the frequency distribution of combined acoustic parameters was not determined. The frequency distribution of the entire piping call, as determined from the combined acoustic parameters, may show a different distribution pattern.

In comparison to the piping call, the contact call of the crimson rosella *P. elegans* was shown to have significant levels of geographic variation, corresponding to the different local populations within subspecies (Ribot et al., 2009), and with genetic differences between some populations (Ribot et al., 2012). Thus, the contact call may be used as a population recognition tool, however this is not supported by playback experiments (Ribot et al., 2013). Crimson rosellas are often observed using piping calls as pairs, with one bird calling, and the other responding (pers. comm. R. Ribot), and it is easy to elicit a response from birds by mimicking their piping call with a whistle (pers. obs.). This may indicate that the piping calls of rosella pairs may converge on a similar call type, as seen in budgerigars (Hile et al., 2000).

4.3 Limitations and future studies

One of the main limitations of this study is that the individuals recorded were unmarked. In order to conclusively determine the function of the piping call, calls should be recorded from known, marked individuals. Unfortunately, due to the nature of the piping call and the crimson rosella, it would be difficult to carry out such a study on free-living birds, without a considerable investment of time and other resources. The use of coloured leg bands or wing tags would enable identification of individual birds, however capturing wild birds could prove a challenge, as rosellas are difficult to catch in mist-nets, and are easily stressed (M. Berg, pers. comm). This study did not investigate possible sex differences in piping calls, as sex of the birds was unknown. While it is difficult to sex crimson rosellas without genetic analysis, this could be achieved by collecting DNA samples at the same time as marking individuals. Condition of recorded birds was unknown, and in order to rectify this in future studies, rosellas could be scored for condition at the time of marking. One major assumption of this study was that each recording contained the calls of only one individual, which may not have always been the case. This problem could be addressed in the future by targeted recording of piping calls of marked individuals.

Another potential limitation is that calls were recorded during only one breeding season, so possible seasonal or temporal changes in the calls of individuals have not been observed. In addition, recordings were only made of single calling bouts, so any short-term changes in the calls of individual rosellas would not be seen. To avoid this in the future, known individuals should be recorded on multiple occasions. In order to fully test for ontogenetic variation, future studies should sample juvenile and subadult age groups in greater numbers, as these groups were not well represented in this study.

Future research should include playback studies between different subspecies and age classes to determine the reactions and responses of individuals to different piping calls, as there was a lack of experimental testing in this study. Such studies have been carried out on many species (Ribot et al., 2013, Vehrencamp et al., 2003, Wright and Dorin, 2001), and enable conclusions about the function of calls to be drawn. The piping call would be suitable for playback experiments as crimson rosellas do respond to pre-recorded playback stimulus, and mimicking the whistle of a piping rosella often elicits a response that continues for multiple calls (pers. obs.). Playback experiments could be in the form of a factorial design playback experiment, to test the responses of birds to their own piping call, and the piping call of their mate and other rosellas in their local population, as well as the piping calls of unfamiliar individuals from different subspecies.

In addition to playback experiments, I suggest a further in-depth analysis of piping calls from known individuals to search for, and identify, potential vocal markers.

4.5 Conclusions

In contrast to previous results in contact calls, crimson rosellas showed little subspecies or age variation in their piping calls, however they do have high levels of individual variation. I suggest that frequency measures are highly important in individual variation amongst crimson rosellas. Further study, including experimental testing and the study of known individuals, is required to conclusively determine the function of the piping call of the crimson rosella, and whether it serves as an individual signalling and recognition tool. This study has provided a starting point for in-depth analysis of the piping call, and has expanded our knowledge of crimson rosella vocalisations.

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Supplementary material

Supplementary table 1. Details of recording sites. Information from (Ribot et al., 2009). All calls recorded by R. Ribot.

Site number	Site	Subspecies	Longitude	Latitude	Number of recording days	Recording dates
1	Deep Creek Conservation Park	*fleurieuensis*	138°11'	35°37'	7	17 Sept - 28 Nov 2004
2	Second Valley Forest Reserve	*adelaidae*	138°17'	35°32'	7	17 Sept - 28 Nov 2004
3	Scott Park	*adelaidae*	138°43'	35°26'	7	17 Sept - 28 Nov 2004
4	Mount Billy Conservation Park	*adelaidae*	138°35'	35°27'	7	17 Sept - 28 Nov 2004
5	Uraidla, SA	*adelaidae*	138°45'	34°58'	16	21 Sept 2004 - 22 Jan 2005
6	Crafers, SA	*adelaidae*	138°42'	35°00'	16	21 Sept 2004 - 22 Jan 2005
7	Old Mt. Barker Rd, Echunga	*adelaidae*	138°48'	35°06'	16	21 Sept 2004 - 22 Jan 2005
8	Pichord Rd, Oakbank	*adelaidae*	138°49'	34°58'	16	21 Sept 2004 - 22 Jan 2005
9	Clare Valley, SA	*subadelaidae + adelaidae*	138°35'	33°54'	2	9 Oct - 13 Nov 2004
10	Chain of Ponds, SA	*adelaidae*	138°51'	34°49'	16	21 Sept 2004 - 22 Jan 2005
11	Cambrai Conservation Park	*adelaidae*	139°22'	34°38'	16	21 Sept 2004 - 22 Jan 2005
12	Cambrai, SA	*adelaidae*	139°16'	34°39'	16	21 Sept 2004 - 22 Jan 2005
13	Flinders Ranges	*adelaidae*	138°00'	32°17'	2	12 Nov - 3 Nov 2004

Site number	Site	Subspecies	Longitude	Latitude	Number of recording days	Recording dates
14	Gundagai, NSW	*elegans x flaveolus*	148˚06'	35˚04'	14	23 Oct - 19 Dec 2004
15	Brungle, NSW	*elegans x flaveolus*	148˚12'	35˚10'	14	23 Oct - 19 Dec 2004
16	Baranduda Reserve	*elegans x flaveolus*	147˚00'	36˚12'	14	23 Oct - 19 Dec 2004
17	Mount Murramurranbong National Park	*elegans*	146˚58'	36˚18'	9	3 Nov 2004 - 15 Jan 2005
18	Bunyip State Park	*elegans*	145˚39'	37˚58'	9	3 Nov 2004 - 15 Jan 2005
19	Strathbogie State Forest	*elegans*	145˚55'	36˚54'	9	3 Nov 2004 - 15 Jan 2005
20	Mount Buffalo National Park	*elegans*	146˚49'	36˚43'	9	3 Nov 2004 - 15 Jan 2005
21	Alpine National Park	*elegans*	147˚12'	36˚34'	9	3 Nov 2004 - 15 Jan 2005
22	Lower Glenelg National Park	*elegans*	141˚08'	38˚01'	9	3 Nov 2004 - 15 Jan 2005
23	Albury, NSW	*elegans x flaveolus*	146˚54'	36˚03'	14	23 Oct - 19 Dec 2004
24	Gunbower State Forest	*flaveolus*	144˚44'	36˚07'	6	9 Nov 2004 - 19 Jan 2005
25	Morgan Conservation Park	*flaveolus*	139˚42'	34˚01'	6	9 Nov 2004 - 19 Jan 2005

Supplementary table 2. Comparisons of ten acoustic variables describing piping calls from four subspecies of the *Platycercus elegans* species complex in southeastern Australia

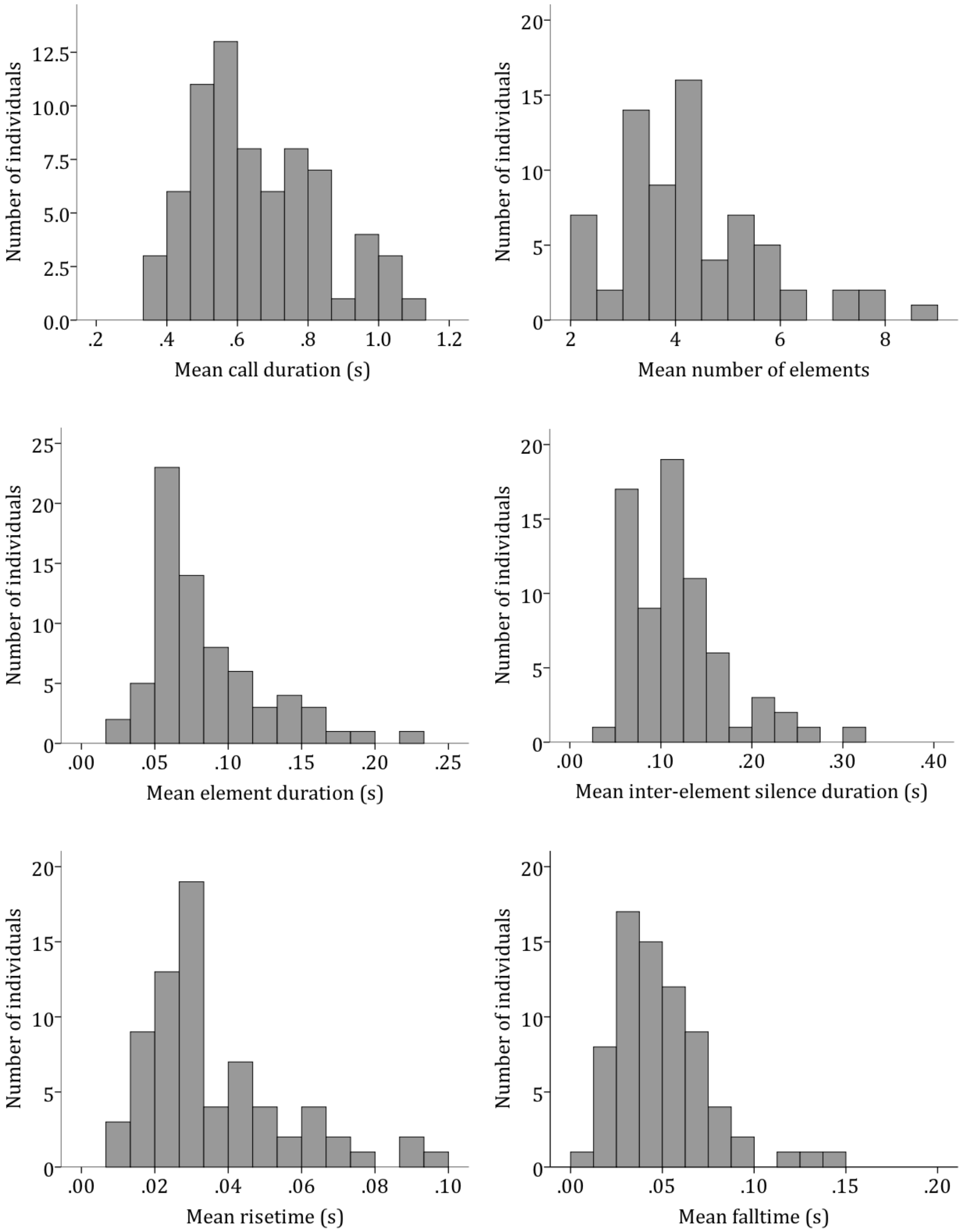
Response variable	**Predictor variable**	**Estimate±SE**	***df***	**Statistic (*F*/*Z*/*t*)**	***p-value***	**ICC**
Call duration (s)	*Fixed*					
	Subspecies		3,18	0.102	0.958	
	Age		2,60	1.053	0.355	
	Julian Day	-0.002±0.001	1,68	3.364	0.071	
	Subspecies * Age		6,49	0.954	0.466	
	Random					
	Individual identity	0.009±0.003		2.806	**0.005**	0.18
	Site	0.018±0.008		2.11	**0.035**	0.34
Number of elements	*Fixed*					
	Subspecies		3,21	1.664	0.205	
	Age		2,72	2.307	0.107	
	Julian Day	0.001±0.006	1,58	0.047	0.830	
	Subspecies * Age		6,66	1.455	0.207	
	Random					
	Individual identity	0.086±0.190		4.462	**<0.001**	0.32
	Site	1.154±0.473		2.44	**0.015**	0.44

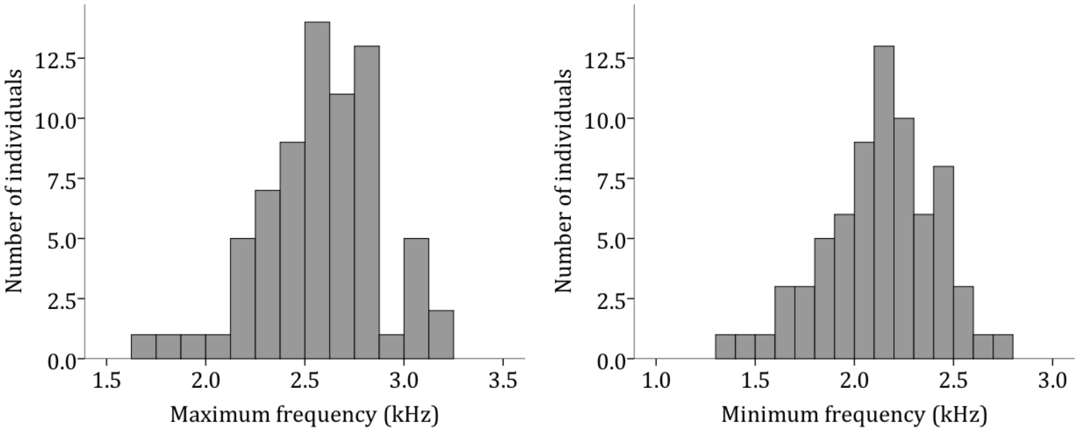
Response variable	**Predictor variable**	**Estimate±SE**	***df***	**Statistic ($F/Z/t$)**	***p-value***	**ICC**
Element duration (s)	*Fixed*					
	Subspecies		3,7	4.022	0.059	
	Age		2,74	0.912	0.406	
	Julian Day	<-0.001±<0.001	1,22	5.993	**0.023**	
	Element number	0.001±0.001	1,1062	0.495	0.482	
	Subspecies * Age		6,70	1.995	0.078	
	Random					
	Individual identity	0.001±<0.001		5.235	**<0.001**	0.41
	Site	0.001±<0.001		1.877	0.061	0.20
Inter-element silence (s)	*Fixed*					
	Subspecies		3,18	0.92	0.451	
	Age		2,69	0.732	0.485	
	Julian Day	<0.001±<0.001	1,39	0.008	0.930	
	Element number	-0.003±0.001	1,773	3.277	0.071	
	Subspecies * Age		6,64	0.751	0.611	
	Random					
	Individual identity	0.001±<0.001		4.459	**<0.001**	0.31
	Site	0.001±<0.001		1.82	0.069	0.17

Response variable	**Predictor variable**	**Estimate±SE**	***df***	**Statistic (*F*/*Z*/*t*)**	***p-value***	**ICC**
Risetime (s)	*Fixed*					
	Subspecies		3,19	2.027	0.145	
	Age		2,68	0.617	0.542	
	Julian Day	<-0.001±<0.001	1,43	0.554	0.461	
			1,106			
	Element number	-0.001±0.001	2	3.921	**0.048**	
	Subspecies * Age		6,63	0.473	0.826	
	Random					
	Individual identity	<0.001±<0.001		4.362	**<0.001**	0.24
	Site	<0.001±<0.001		2.099	**0.036**	0.19
Falltime (s)	*Fixed*					
	Subspecies		3,77	3.424	**0.021**	
	Age		2,75	0.558	0.575	
	Julian Day	<-0.001±<0.001	1,83	9.991	**0.002**	
			1,106			
	Element number	-0.001±0.001	5	2.777	0.096	
	Subspecies * Age		6,75	2.853	**0.015**	
	Random					
	Individual identity	0.001±<0.001		5.956	**<0.001**	0.49

Response variable	**Predictor variable**	**Estimate±SE**	***df***	**Statistic (*F*/*Z*/*t*)**	***p-value***	**ICC**
Maximum frequency (Hz)	*Fixed*					
	Subspecies		3,75	1.347	0.266	
	Age		2,74	1.783	0.175	
	Julian Day	1.002±1.105	1,82	0.822	0.367	
	Element number	-7.889±2.547	1,858	9.591	**0.002**	
	Subspecies * Age		6,73	0.512	0.798	
	Random					
	Individual identity	74104.403±12960.758		5.718	**<0.001**	0.62
	Site	8104.275±8503.614		0.953	0.341	0.07
Minimum frequency (Hz)	*Fixed*					
	Subspecies		3,4	0.922	0.502	
	Age		2,76	1.664	0.196	
	Julian Day	0.795±1.151	1,22	0.477	0.497	
	Element number	-2.402±1.889	1,857	1.617	0.204	
	Subspecies * Age		6,71	0.486	0.817	
	Random					
	Individual identity	63828.293±12413.589		5.142	**<0.001**	0.61
	Call identity	25923.840±2786.801		9.302	**<0.001**	0.25
	Site	7952.495±8088.936		0.983	0.326	0.07

Response variable	**Predictor variable**	**Estimate±SE**	***df***	**Statistic (*F*/*Z*/*t*)**	***p-value***	**ICC**
Peak frequency (Hz)	*Fixed*					
	Subspecies		3,6	1.259	0.368	
	Age		2,75	1.743	0.182	
	Julian Day	0.642±1.052	1,26	0.372	0.547	
	Element number	-6.672±2.201	1,863	9.185	**0.003**	
	Subspecies * Age		6,71	0.555	0.765	
	Random					
	Individual identity	51778.089±10312.875		5.02	**<0.001**	0.56
	Call identity	24209.661±2693.938		8.987	**<0.001**	0.26
	Site	6915.966±6520.404		1.061	0.289	0.07
Frequency bandwidth (Hz)	*Fixed*					
	Subspecies		3,73	0.493	0.688	
	Age		2,72	1.428	0.247	
	Julian Day	0.238±0.854	1,77	0.077	0.782	
	Element number	-5.224±2.043	1,867	6.536	**0.011**	
	Subspecies * Age		6,72	0.136	0.991	
	Random					
	Individual identity	37068.354±6506.156		5.697	**<0.001**	0.67
	Call identity	9378.858±1215.450		7.716	**<0.001**	0.17


Number of individuals
12.5
10.0
7.5
5.0
2.5
0.0
.2
.4
.6
.8
1.0
1.2
Mean call duration (s)
Number of individuals
20
15
10
5
0
2
4
6
8
Mean number of elements
Number of individuals
25
20
15
10
5
0
.00
.05
.10
.15
.20
.25
Mean element duration (s)
Number of individuals
20
15
10
5
0
.00
.10
.20
.30
.40
Mean inter-element silence duration (s)
Number of individuals
20
15
10
5
0
.00
.02
.04
.06
.08
.10
Mean risetime (s)
Number of individuals
20
15
10
5
0
.00
.05
.10
.15
.20
Mean falltime (s)



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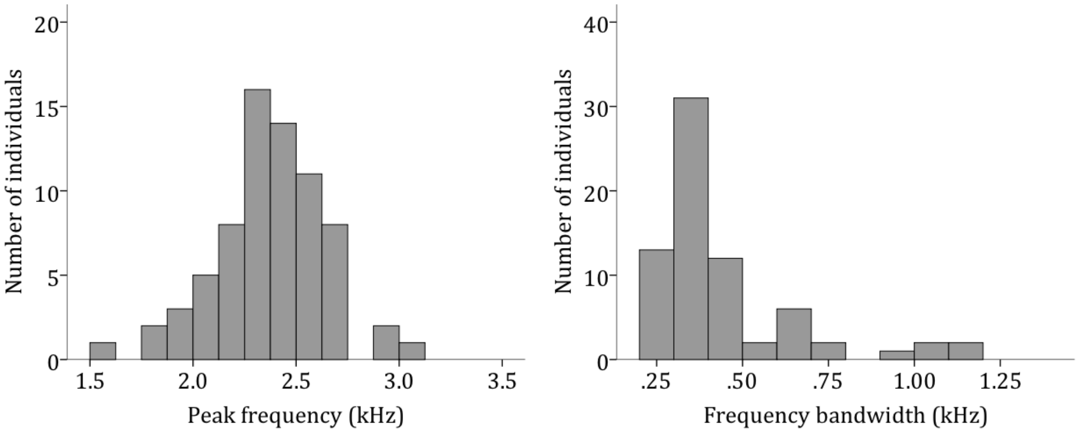

Supplementary table 1. Frequency histograms of acoustic variables describing piping calls from four populations of *Platycercus elegans* species complex in southeastern Australia. N = 89 individuals. See text for definition of acoustic variables.