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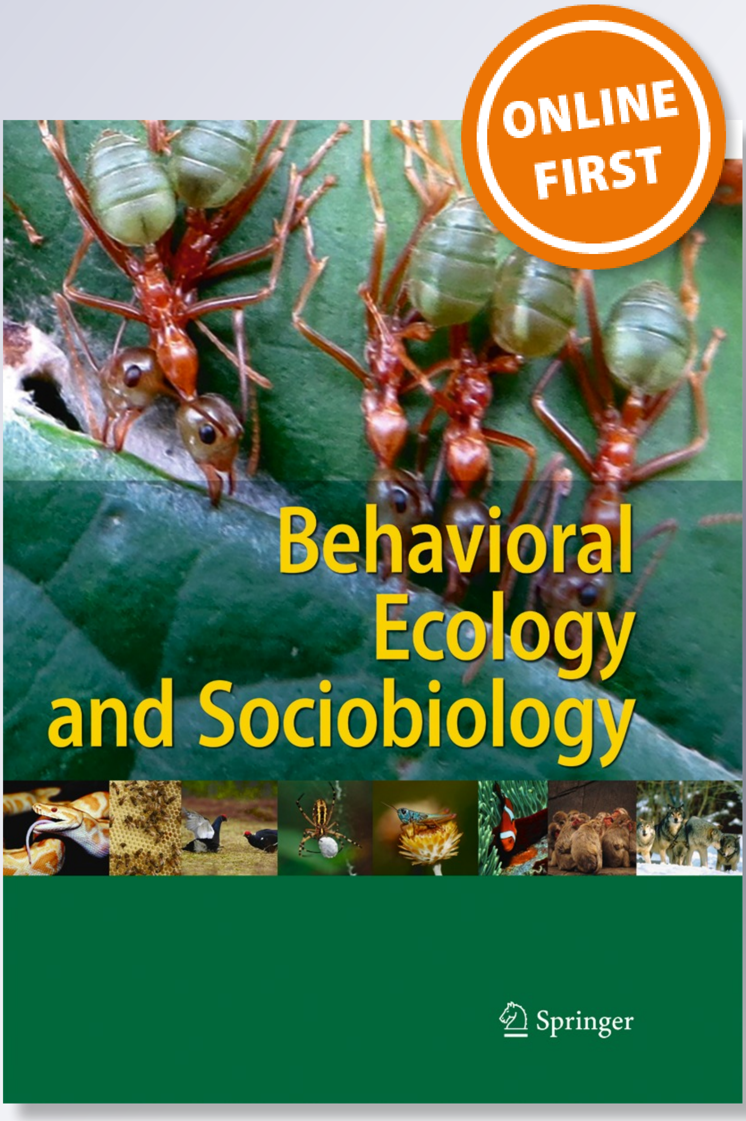

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ORIGINAL PAPER

Geographic variation in aggressive signalling behaviour of the Jacky dragon

Marco D. Barquero[1,2] · **Richard Peters**[3] · **Martin J. Whiting**[1]

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Abstract Signal diversification is often the product of sexual and/or natural selection and may be accompanied by genetic differentiation or simply reflect a plastic response to social and environmental variables. We use an agamid lizard endemic to Australia, the Jacky dragon (*Amphibolurus muricatus*), to examine the relationships between population relatedness, morphology and signalling behaviour. We also tested whether males are able to discriminate among rivals from different populations and whether they respond more aggressively to more closely related populations. We studied three populations, two of which belong to the same genetic clade. Individuals from the two most closely related populations were also more similar in morphology than lizards from the third, more distant, population. However, all three populations differed in characteristics of their signalling behaviour including latency to display and the interval between displays. In addition, animals from all populations showed similar levels of aggression when matched with individuals from the same or different populations in staged trials and thus did not show evidence of population-level discrimination. We argue that display variation might be a consequence of behavioural plasticity and that, despite difference in genetic structure, morphology and behaviour, this species retains a cohesive communication system.

Keywords *Amphibolurus* · Australia · Lizard · Population variation · Visual displays

Animal communication is a rich and challenging field in large part because of the immense diversity of signals in a wide range of taxa. In the case of lizards, visual displays have gained attention as a highly variable communicative system (Persons et al. 1999). Lizards can signal using colour patches, body posturing and dynamic visual signals such as tail flicks, leg waves and gular or dewlap extensions (Carpenter and Ferguson 1977; Jenssen 1977). Across-species variation is well known, and both natural and sexual selection have been invoked to explain signal diversity in a phylogenetic context (Ord et al. 2002; Stuart-Fox et al. 2007). However, intraspecific variation has received much less attention and relatively few studies have accounted for variation in visual signal structure across populations of the same species (e.g. Ferguson 1971; Jenssen 1971; Martins et al. 1998; Leal and Fleishman 2004; Bloch and Irschick 2006). While we predict that wide-ranging and ecologically variable species will show high variation in signal repertoire (Ord et al. 2002), this remains to be tested. Furthermore, we know little about signal divergence in widely distributed species and whether they respond differently to individuals from geographically disparate populations. In some species, a stronger response is elicited from individuals in a receiver's neighbourhood compared to more distant, less closely related individuals (Bensch et al.

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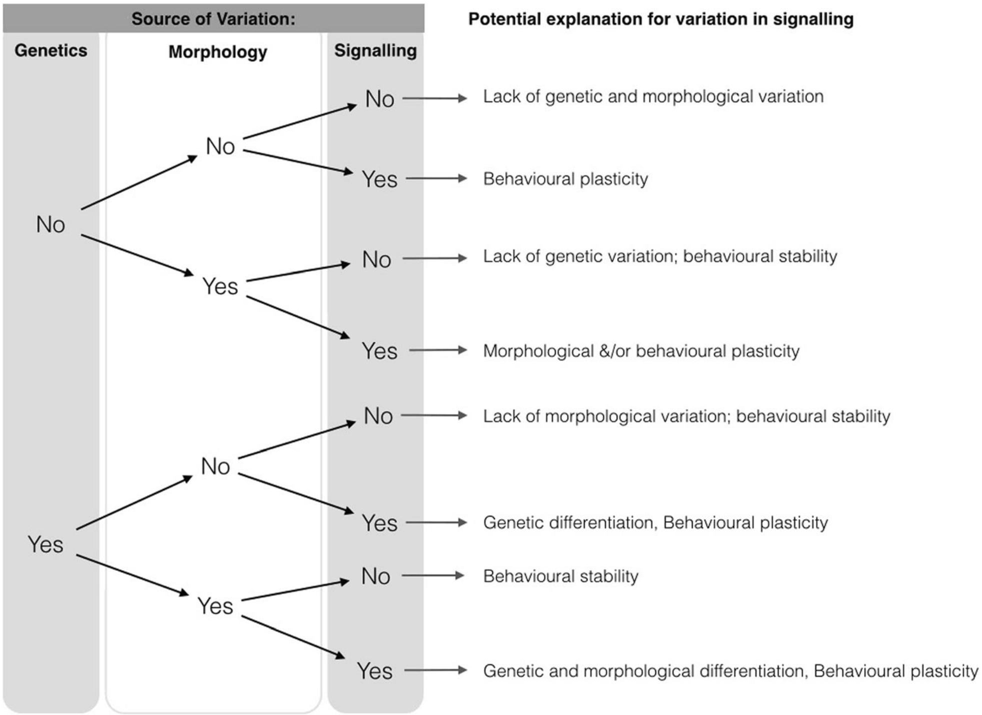
1998; Dunbrack and Clarke 2003). This may be because they constitute less of a threat, possibly as a result of divergence.

The signalling environment can vary dramatically for widely ranging species as a result of habitat structure, environmental noise and predation risk, such that differences in visual signals among populations could be a result of local adaptation (Podos 2001). However, differences in signalling behaviour across the range of a species could also emerge as a result of differences in female preferences during sexual selection (Uy and Borgia 2000) or through a non-adaptive process such as genetic drift (Hill 1994). Whether a trait is the result of genetic divergence is of key importance in understanding how signals evolve. For example, if signal expression is not selected for and it is only the result of genetic differences among populations of the same species, then dispersing animals would not be as constrained by the conditions present at different habitats to perform their visual displays. At the same time, if the variation in genetic structure and signal repertoire is high across the range of a species, then dispersing animals could face problems conveying a message to local individuals since signals might not be recognized. Therefore, despite the opportunity to display freely under different environmental conditions, reproductive isolation could arise if populations generate different signals and species recognition cues are incongruent (Bensch et al. 1998; Leal and Fleishman 2004).

Visual signals may also be constrained by morphological traits of the individuals in a population (Podos 2001). If the morphology used to produce visual signals differs among populations of the same species, then a concordant pattern of variation in the signalling behaviour is predicted. For instance, if the tail of an animal is used to communicate with conspecifics and tail length varies among populations, then some properties of the signal, such as duration and frequency, could also be divergent (Barnard 1991; Young et al. 1994). However, variation in signalling behaviour is not always explained by variation in morphology (Ferguson 1971; Irwin et al. 2008). Therefore, when comparing populations of the same species, it is important to consider how variations in relatedness, morphology and signalling behaviour are related. Figure 1 shows the outcome and associated explanation of the geographic variation in signalling behaviour when morphology and relatedness are considered, but not environmental factors.

Here, we test whether variation in morphology and signalling behaviour among populations of the Jacky dragon (*Amphibolurus muricatus*) correlates with their genetic differentiation. We predicted that greater divergence in genetic structure between populations should produce the same effect in morphology and signalling behaviour. In light of this variation, we also asked whether individuals have population-specific responses and predicted that they would show stronger (more aggressive) visual displays to individuals from their own population. This should manifest itself in latency to display and the frequency of their signals. Our rationale is that animals will display less aggressively to signals with fewer

Fig. 1 Molecular, morphological and signalling behaviour variation when populations of the same species are compared. Variation, when present, can be seen as genotype (or phenotype or behaviour type) 1 in population A, genotype 2 in population B and so on. A potential explanation of the variation in signalling behaviour as a function of molecular and morphological variation is included



familiar components than they would to familiar signals (although see Dunbrack and Clarke 2003).

Methods

Study animal

Jacky dragons are a good model system because they are widely ranging and relatively abundant (Cogger 2000) and their signalling behaviour has been described in detail (Carpenter et al. 1970; Peters and Ord 2003; Watt and Joss 2003). Animals reach sexual maturity at a snout-vent length of 72 mm and can grow up to 120 mm (Harlow and Taylor 2000), with a tail that is 1.5–2 times their body size. Their dorsal colouration ranges from pale grey to dark brown with two light stripes on each side (Cogger 2000). The reproductive season of the species is from September to March, with a hibernation period from May to August (Harlow and Taylor 2000). Jacky dragons use motion-based signals to communicate between conspecifics, and males have readily identifiable aggressive and submissive signals. Aggressive displays consist of five components performed in a stereotypical fashion: an introductory tail flick, a backward foreleg wave, a forward foreleg wave, a push-up and a body rock (Peters and Ord 2003). Although they are only part of the aggressive display of this species, tail flicks have been considered an introductory movement to attract the attention of conspecifics (Peters and Evans 2003) and exhibit situation-specific variation in expression (Peters et al 2007). Consequently, we considered tail flicks separately and use aggressive display (AD) to refer to the remaining motor patterns. The submissive display consists of a slow circumduction of one of the forelimbs.

The Jacky dragon is an endemic Australian dragon lizard extending over 2000 km along the eastern and south-eastern coast of the country. Despite its large distribution and well-documented signalling behaviour, geographic variation of the aggressive and submissive displays has never been measured in this species. Moreover, the Jacky dragon was one of the first Australian reptiles to be described (White 1790) and it has been extensively studied under different biological contexts, yet genetic variation along its range has only recently been reported (Pepper et al. 2014). Molecular analyses revealed the existence of five clades along the distribution of the species, although these were not supported by morphological data (Pepper et al. 2014).

Study populations and captive animals

We visited three sites along the range of the species: Yarratt State Forest (31° 48′ 17.4″ S, 152° 25′ 57.5″ E), Royal National Park (34° 04′ 49.7″ S, 151° 05′ 39.6″ E) and Cann River State Forest (37° 36′ 25.9″ S, 149° 09′ 05.8″ E), henceforth referred to as Yarratt, Royal and Cann River, respectively. Animals from two populations, Yarratt and Royal, are members of the same genetic clade, whereas lizards from Cann River are genetically more distant (see below) (Pepper et al. 2014). Ten to fifteen males were collected from each population and transported to Macquarie University, where they were housed individually in tubs ($70L \times 49W \times 30H$ cm) inside a room maintained at 25 °C and a 12-h light-dark cycle. Individuals were kept in these indoor tubs for 3–4 weeks as a quarantine period before being moved to outdoor pens. The outdoor enclosures ($180L \times 180W \times 88H$ cm) were made from metal sheets, and a divider (45 cm high) was used to split them in half. Each half consisted of a sand substrate, branches suitable for basking, screen for sun cover and plastic containers as refuges and water receptacles. Lizards were fed twice weekly with crickets dusted with calcium and vitamin supplements (Repti-Vite® and Repti-Cal®) and mealworms, and water was provided ad libitum. The Macquarie University's Animal Ethics Committee (ARA 2010/034), the National Parks and Wildlife Service of New South Wales (permit number: S13197) and Department of Environment and Primary Industry, Victoria (permit number: 10005522), approved all housing and experimental procedures.

Genetic relatedness and morphological variation

Using the uncorrected genetic distances generated by Pepper et al. (2014) for two mitochondrial DNA (mtDNA) genes (ND2 and ND4) and three nuclear DNA (nDNA) genes (RAG1, PRLR and BDNF), we established the genetic relatedness among our study populations by calculating pairwise average distances. We used these averages to generate separate distance matrices for mtDNA genes combined and nDNA genes combined.

We examined morphological variation by collecting the following morphometric data from males caught in the field: snout-vent length (SVL), tail length, inter-limb length, head length, width and height, forelimb (length of humerus, length of radius and total length), hindlimb (length of femur, length of fibula and total length) and mass. All morphometrics, except head width, were taken on the right side of the animal. SVL, tail length and total lengths of both limbs were measured with a ruler (to the nearest 0.5 mm), whereas mass was measured with a digital balance (to the nearest 0.1 g) and the remaining measurements with digital calipers (to the nearest 0.1 mm). We also recorded the number of femoral and anal pores from both sides (right and left) of an animal's body.

Display-action-patterns

In order to quantify geographic variation in signal structure, we created display-action-pattern (DAP) profiles for each lizard. Each captive male was transferred to an indoor aquarium

($91L\times35W\times38H$ cm) containing a wooden block as perch site, a mirror at one of the sides and a ruler placed vertically in one of the sides of the aquarium. A heat lamp was suspended above the aquarium, and the walls of the aquarium were covered with paper to avoid disturbing the subject, with one side having a viewing window for filming (Online Resource 1). The mirror was covered when a subject was placed inside the aquarium and was uncovered after 10 min, leaving enough time for the animal to first warm-up. Each subject was filmed one to three times for up to 60 min each time using a Panasonic HDC-HS60-K digital video camcorder mounted on a Manfrotto® tripod. Thirty-five individuals (12 from Cann River, 12 Royal and 11 Yarratt) were tested during 94 sessions. A total of 165 displays were analyzed using custom-written code in Matlab (R. Peters, La Trobe University), tracking the eye of an individual in each frame of a video to define a DAP and using the ruler to scale measurements (pixels to mm). Our approach focuses on the movement of the head during the forward foreleg wave, the push-up and body-rock motor patterns (the head is stationary during the backward foreleg wave). The following measurements were computed as illustrated in Fig. 2: drop in height of the head during the forward foreleg wave (descent amplitude) and the duration of time that the head is held in this lowered position (descent hold time), push-up displacement and body-rock duration (see also Online Resource 1).

Male-male contests and video analysis

Trials were carried out during summer (January and February) of 2012 and 2013, at least 4 months after collection of individuals. Two captive males from the same (intra-population contests) or different (inter-population contests) populations were transferred to a neutral arena similar to the outdoor enclosures described above, but containing only one T-shaped wooden block (50 cm high) as a perch site. One or both individuals were temporarily marked with a xylene-free paint pen and then placed at the same time within the arena. Interactions were filmed for up to 90 min using a Panasonic HDC-HS60-K digital video camcorder mounted on a Manfrotto® tripod. The equipment was placed behind a thick black mat to avoid disturbing the subjects. Although not in consecutive sessions or days, each captive male had one to four intra-population and one to two inter-population encounters with different opponents and a total of 88 sessions were recorded. The neutral arena was sprayed with water and swept after each session in order to reduce the likelihood of chemical signals interfering with the behaviour of a new pair of subjects. In addition, we performed an analysis of covariance, with the log-transformed number of substrate snout-touches as the dependent variable, population as a fixed factor and log-transformed number of ADs as a covariate. We did not find significant differences in the number of substrate snout touches across populations ($F_{2,35}=2.75$, $P=0.078$).

Videos were analyzed using JWatcher Video version 1.0 as an event recorder program (Blumstein and Daniel 2007), scoring the following behaviours: tail flick (TF), aggressive display (AD), submissive display (SD), bite attack (BA, lizard lunges towards an opponent with an open mouth), chase (C) and dorsal grasp (DG, defined as an individual grasping another from the back). All videos were viewed twice, scoring the behaviours for one subject at a time. The following information was then obtained for all the contests in which each animal participated: average latency to the first TF, AD and SD; average number of TFs, ADs and SDs; and average duration of inter-TF, inter-AD and inter-SD intervals. Inter-display intervals were calculated as the time elapsed between the start of two behaviours (e.g. time between AD1 and AD2). Because it has been demonstrated that TFs and ADs in the Jacky dragon usually occur in bouts and pauses between bouts can last for 6–12 s (Ord and Evans 2003), we also calculated the average number of bouts and the average inter-bout interval.

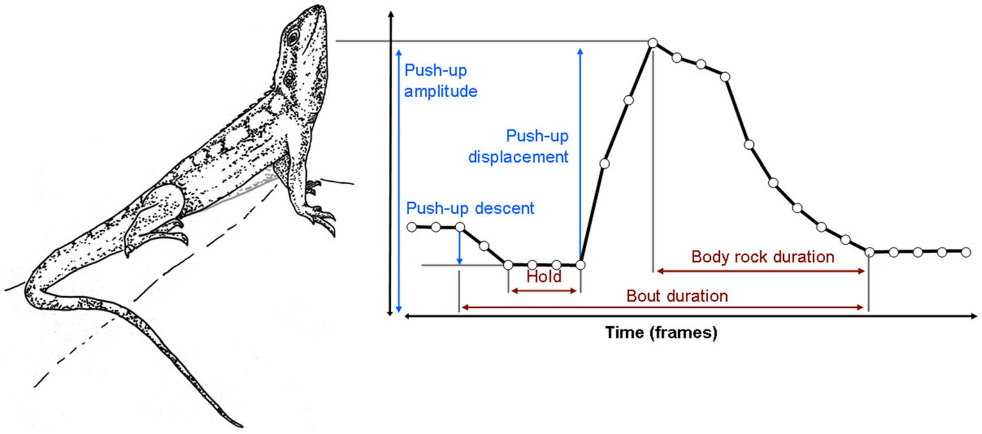


Fig. 2 Sequence of the movements involved in the push-up display of the Jacky dragon (drawing by Jose Ramos)

Discrimination of populations

To determine whether animals behaved differently to conspecifics from their own population relative to conspecifics from different populations, we created an index of aggression weighting each behaviour described above as follows, in order of increasing aggressiveness: TF 1, AD 2, DG 3, C 4 and BA 5. Because individuals participated in up to three intra-population contests, we used an average score of these contests for each animal. We also used separate scores for each animal during inter-population contests (e.g. one animal from Cann River had a score for a contest against an animal from Royal and another score for a contest against an animal from Yarratt).

Statistical analysis

In order to quantify variation in morphology across populations of the Jacky dragon, we used 13 morphometric and two meristic traits collected on adult males in the field. Both sets of variables were analyzed separately. First, we regressed all morphometric variables against SVL to remove the effect of size and then used the residuals to perform a multivariate analysis of variance (MANOVA) with population as a fixed factor. Bonferroni post hoc tests were used to determine which populations differed. We compared the average number of femoral and anal pores across populations using separate Kruskal-Wallis tests.

From the data used to create the DAP graphs, we performed linear mixed effects models from the *lme4* package (Bates et al. 2014) in R 2.13.0, with population as a fixed effect and lizard identity as a random effect to control for multiple observations from the same lizard. We determined the significance of the full model for each variable as well as investigating all pairwise comparisons. To analyze the signalling behaviour of the Jacky dragon, we first tested their willingness to display (i.e. number of displaying and non-displaying animals) either aggressively or submissively within each population using separate binomial tests for each population. We also determined whether variation in characteristics of the signalling behaviour occurs among populations. From the data generated during the male-male contests, for each behavioural variable, we first averaged the data of all encounters in which a lizard was used and then log-transformed only those variables (average number of TFs, ADs, SDs and bouts) that did not fit a normal distribution. Four individuals (one from Cann River, one from Royal and two from Yarratt) produced no displays of any kind and were excluded from further analyses. We then performed a MANOVA on the 11 behavioural variables using population as a fixed factor. Bonferroni post hoc tests were used to determine which populations differed from each other.

We were interested in determining the relative contribution of morphological and behavioural traits and whether they could be used to classify animals according to population. We first removed the effect of size by regressing all morphometric variables against SVL and then using the residuals. We then reduced the number of variables (13 morphometric and 11 behavioural extracted from the male-male contests) by performing a factor analysis with principal components and varimax as the extraction and rotation methods, respectively. We used the scores of the factors (principal components [PCs]) extracted as predictor variables in a discriminant function analysis (DFA), with population as the grouping variable.

We used an analysis of covariance to determine whether the levels of aggression among populations differed. We averaged the scores of the aggression index and used the log-transformed variable as the dependent variable. We also averaged the scores and the number of SDs of the rivals that each animal confronted and used the log-transformed variables as covariates. The population of the individual and population of the rival were used as fixed factors. Unless otherwise specified, all analyses were carried out using SPSS Statistics version 20.0 (IBM©).

Results

Genetic and morphological variation

We found higher average genetic distances for both mtDNA genes and nDNA genes, when individuals from Royal and Yarratt were compared to those from Cann River. Comparisons between Royal and Yarratt yielded similar averages (Table 1). We found significant differences for most morphometric traits (e.g. tail length, head dimensions and limb measurements) and for both femoral and anal pores (Table 2 and Online Resource 2) across populations. Post hoc tests revealed that animals from Cann River differed significantly from those of Yarratt and Royal for most of the morphological traits (Table 2). Animals from Royal and Yarratt differed only in the relative length of the hindlimb, with lizards of the latter having longer hindlimbs (Table 2).

Variation in signalling behaviour

In terms of AD signal structure obtained from DAP profiles, we found differences among populations in the descent amplitude ($F_{2,10}$=5.87, P=0.021) and body-rock duration ($F_{2,10}$=5.38, P=0.026). Pairwise comparisons showed that individuals from Yarratt performed displays with smaller amplitudes (t=−2.95, P=0.015) and shorter body-rocks (t=−3.24, P=0.009) than those from Royal, whereas individuals from Royal showed bigger amplitudes than lizards from Cann River (t=3.08, P=0.012) (Fig. 3).

Table 1 Uncorrected P distance matrices for three populations of the Jacky dragon showing the averages of two mtDNA genes and three nDNA genes

mtDNA	Cann River	Royal	Yarratt
Cann River	0.0011 (0–0.0029)		
Royal	0.0280 (0.0229–0.0304)	0.0035 (0–0.0081)	
Yarratt	0.0298 (0.0281–0.0315)	0.0067 (0.0026–0.0092)	0.0040 (0–0.0080)
nDNA	Cann River	Royal	Yarratt
Cann River	0.0004 (0–0.0008)		
Royal	0.0014 (0.0004–0.0033)	0.0015 (0–0.0034)	
Yarratt	0.0017 (0.0004–0.0029)	0.0015 (0.0004–0.0032)	0.0009 (0.0004–0.0020)

Range (min-max) shown in parentheses (Pepper et al. 2014)

Animals from the three populations differed in their willingness to display, either aggressively or submissively, towards conspecifics during male-male contests. Similar proportions of contests with and without displays were observed for lizards from Cann River and Royal during intra- and inter-population contests (Table 3). Animals from Yarratt produced less ADs in both types of contests (Table 3). Differences between populations were also detected in all behavioural traits extracted from male-male contests (Table 4). Overall, animals from Cann River started to display sooner and produced more displays and bouts with shorter inter-display intervals compared to lizards from Yarratt (Fig. 4). Cann River and Royal were not significantly different for any display variable. Individuals from the latter population produced ADs and SDs sooner and with shorter inter-display intervals than those from Yarratt (Fig. 4).

Table 2 Results of the statistical tests applied to morphometric (univariate Fs extracted from a MANOVA) and meristic (chi-square extracted from Kruskal-Wallis tests) traits to compare between three populations of the Jacky dragon

Morphometric trait	$F_{2,81}$	*P* value	CR vs R	CR vs Y	R vs Y
Tail	16.08	*<0.001*	*<0.001*	*<0.001*	1.000
Inter-limb	0.14	0.870	1.000	1.000	1.000
Head length	12.37	*<0.001*	*<0.001*	*0.006*	0.929
Head width	4.73	*0.011*	1.000	*0.011*	0.062
Head height	2.98	0.056	0.167	1.000	0.088
Humerus	6.61	*0.002*	*0.009*	*0.010*	1.000
Radius	11.94	*<0.001*	*<0.001*	*0.001*	1.000
Forelimb	3.20	*0.046*	1.000	*0.047*	0.162
Femur	8.97	*<0.001*	*0.003*	*0.001*	1.000
Fibula	28.71	*<0.001*	*<0.001*	*<0.001*	1.000
Hindlimb	38.48	*<0.001*	*<0.001*	*<0.001*	*0.013*
Mass	4.23	*0.018*	0.880	*0.014*	0.188
Meristic trait	$X^2(df=2)$	*P* value	CR	R	Y
Femoral pores	38.36	*<0.001*	61.25	28.10	29.43
Anal pores	8.86	*0.012*	49.59	35.33	38.78

P values of pairwise post hoc tests using Bonferroni are also shown for morphometric traits. Ranks for each population are shown for each meristic trait. Statistically significant results are shown in italics

CR Cann River ($n=35$), *R* Royal ($n=29$), *Y* Yarratt ($n=20$)

Morphological and behavioural divergence

The factor analysis identified five significant PCs (eigenvalues ≥1) accounting for 83.23 % of the total variance. Morphometrics loaded more strongly on PCs 2, 4 and 5, while behavioural variables loaded strongly on PCs 2 and 3. The DFA produced two significant discriminant functions (function 1: Wilks' lambda=0.26, $X^2=41.49$, $df=10$, $P<0.001$;

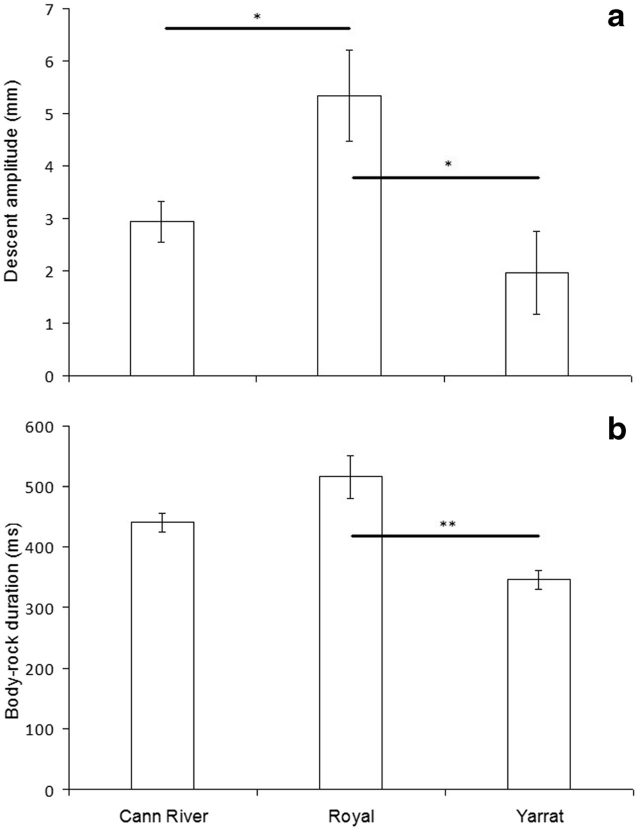


Fig. 3 Two characteristics (mean±standard error) of the push-up display of the Jacky dragon compared across three populations. Significant results among pairwise comparisons are shown (*$P<0.05$, **$P<0.01$)

Table 3 Binomial tests (*P* values) comparing the number of contests (either intra- or inter-population) in which Jacky dragons of three populations performed aggressive or submissive displays according to contest type (intra- or inter-population) and irrespective of the origin of the competitor (total)

Population	Contest type		Total
	Intra-population	Inter-population	
Aggressive displays			
Cann River	0.405	0.152	0.092
Royal	0.405	0.832	0.358
Yarratt	0.065	0.052	*0.005*
Submissive displays			
Cann River	0.132	0.839	0.366
Royal	0.868	0.832	1.000
Yarratt	0.065	0.286	*0.025*

Significant values are in italics

function 2: Wilks' lambda=0.70, X^2=10.92, *df*=4, P=0.028) that classified correctly 69.4 % (Fig. 5) of individuals by population (83 % for Cann River, 58 % for Royal and 67 % for Yarratt).

Discrimination of populations

Despite differences in genetics, morphology and signalling behaviour, we found no significant differences in the overall levels of aggression for animals from each population ($F_{4,94}$=0.64, P=0.632).

Table 4 MANOVA (univariate Fs) and pairwise post hoc tests using Bonferroni (*P* values) performed on behavioural traits comparing between three populations of the Jacky dragon

Trait	$F_{2,33}$	*P* value	CR vs R	CR vs Y	R vs Y
Latency TF	6.60	*0.004*	0.240	*0.003*	0.231
Latency AD	12.13	*<0.001*	0.279	*<0.001*	*0.011*
Latency SD	13.76	*<0.001*	0.531	*<0.001*	*0.002*
Inter-TF interval	7.41	*0.002*	0.109	*0.002*	0.321
Inter-AD interval	9.70	*<0.001*	0.396	*<0.001*	*0.025*
Inter-SD interval	11.41	*<0.001*	0.596	*<0.001*	*0.007*
Inter-bout interval	10.53	*<0.001*	0.169	*<0.001*	*0.042*
Log no. TFs	4.68	*0.016*	0.122	*0.017*	1.000
Log no. ADs	4.89	*0.014*	0.273	*0.011*	0.530
Log no. SDs	6.02	*0.006*	1.000	*0.006*	0.059
Log no. bouts	4.62	*0.017*	0.235	*0.015*	0.714

Statistically significant results are shown in italics

CR Cann River (*n*=12), *R*=Royal (*n*=12), *Y* Yarratt (*n*=12)

Discussion

Our study confirmed that genetic, morphological and behavioural variations occur in the three populations of *A. muricatus*. However, contrary to our prediction, genetic similarity resulted in similar morphology but not in signalling behaviour despite males from each population showing similar levels of aggression towards rivals. Our results showed that body dimensions were similar for individuals from two closely related populations (Royal and Yarratt) but differed significantly from a more distant population (Cann River). This pattern, however, was not concordant with variation in signalling behaviour, since all three populations differed in the latencies and time elapsed between displays performed during agonistic interactions.

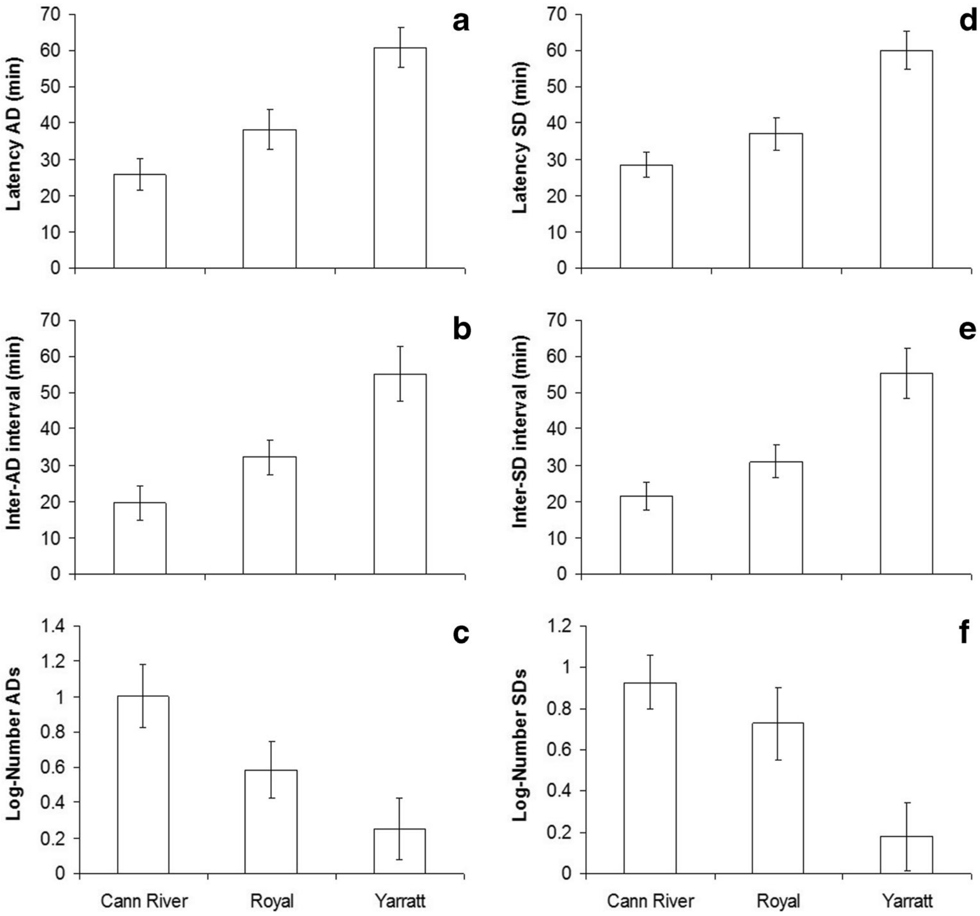
Interaction of genetics, morphology and behaviour

Patterns of genetic, morphological and signal divergence, although essential for understanding the consequences for processes like speciation, are not commonly addressed together in a single study, especially for motion-based signals. Molecular and morphological divergence is commonly observed in widely ranging species, with allopatric populations showing higher levels of differentiation (Irwin et al. 2008). Variation in signalling behaviour is more contentious. Both signal lability (Bloch and Irschick 2006) and stability (Lovern et al. 1999) have been reported for species with wide distributions or low gene flow between populations. Genetic, morphological and signal divergence all occurs across the sampled range of the Jacky dragon, although in a discordant pattern.

We found that populations that are genetically more closely related (Royal and Yarratt) were also more similar in morphology compared to a genetically more distant population (Cann River). Because the phenotypic characteristics that we measured are used to produce signals (i.e. display morphology), a concordant pattern of variation would reflect more similarities between Royal and Yarratt in the signalling behaviour and different characteristics of the visual displays for animals from Cann River. However, Cann River and Royal were more similar in both temporal (e.g. latency to display, inter-display interval, body-rock duration) and structural (e.g. descent amplitude) characteristics of the visual displays compared to Yarratt. This discordant pattern could be a reflection of behavioural plasticity in this species.

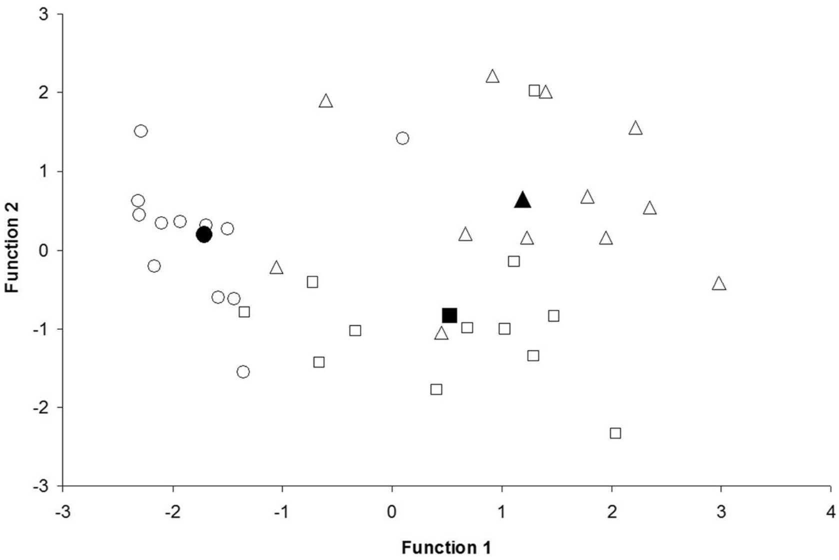
Behavioural plasticity can be the result of differences in selective forces across the range of a species (Snell-Rood 2013). Indeed, habitat characteristics have been commonly used to explain geographic differences in the expression of signals between populations of the same species (Leal and Fleishman 2004; Endler et al. 2005; Bloch and Irschick 2006). Previous work on the Jacky dragon (Peters et al. 2007; Peters 2008) and other lizards (Fleishman 1992; Ord

Fig. 4 Behavioural characteristics (mean±standard error) of three populations of the Jacky dragon. Characteristics of **a**–**c** the aggressive display (AD) and **d**–**f** the submissive display



et al. 2007) has shown that environmental noise, specifically plant motion, can interfere in the effective transmission of motion-based signals. However, other factors can also be involved in shaping the diversification of signals, such as predation pressure (Stuart-Fox et al. 2003; Steinberg et al. 2014) and differences in density among populations (Bloch and Irschick 2006). For example, by targeting animals performing conspicuous displays, predators can change the display rates observed in a population (Endler 1987). Also, in denser populations, the number of displays can increase due to the proximity of individuals and higher encounter rates (Birkhead 1978). Both factors

Fig. 5 Discriminant scores based on morphological and behavioural variables collected from individuals of three populations of the Jacky dragon (*open circles* Cann River, *open squares* Royal, *open triangles* Yarratt). *Solid shapes* represent centroids of each population



may have an effect on the average phenotype found in a population, and therefore, geographical changes of the signalling behaviour might be uncorrelated with changes in display morphology (Wiens 2000).

Interestingly, we found that animals from Yarratt were less willing to display, either aggressively or submissively and took longer to perform the first display and subsequent displays, and overall, their displays were characterized by lower amplitudes and shorter durations. Research on variation in habitat characteristics, predation risk and population density will help to determine the cause of the discordant pattern observed between genetics, morphology and signalling behaviour in the Jacky dragon.

Implications of signal variation

Geographic changes in the signalling behaviour of a species can have significant effects on communication between individuals from different populations. For example, it can be a factor causing reproductive isolation, when signals of dispersing animals are not recognized by local conspecifics, or it can change the preferences of mates when ‘novel’ courtship signals are favoured. In the case of the Jacky dragon, our study revealed significant geographic variation in signalling behaviour. The variation observed occurred on some temporal and structural characteristics of the core display of the species, which could be affected by changes in environmental conditions. However, the components involved in the displays and the sequence of these components were still highly stereotyped across populations, revealing that these signals must have an underlying genetic basis. Therefore, the signalling behaviour of the Jacky dragon is another example of a species retaining an ancestral motor pattern that has been modified along the range of the species possibly by behavioural plasticity (Foster 1999).

Despite the strong effect of selection as a driver of signal divergence in many species, reproductive isolation and changes in mate preferences can be caused by other factors, such as adaptive differentiation in morphology. For example, Jacky dragons flick their tails as an introductory signal to get the attention of conspecifics. Differences in tail length were found among our study populations, but these differences could simply be the result of selection acting on habitat use, for instance, and not to facilitate more effective communication within each population. Two questions emerge: (1) to what extent modifications in signalling behaviour will effect communication among conspecifics from different populations? (2) Are differences in morphology stronger than those of signalling behaviour as a cause of population divergence?

Our results show that despite population differences in display behaviour, similar levels of aggression occur between individuals of the same and different populations. This suggests that species and mate recognition systems are conserved and that Jacky dragons have retained cohesion in terms of visual communication. Also, we found a similar pattern of population divergence when morphological and behavioural traits were considered together compared to the pattern when morphology was considered by itself (Fig. 5). This indicates that differences in morphology might have a stronger influence in the direction of selection.

In summary, divergence in signalling behaviour has been commonly invoked as an important factor causing reproductive isolation and speciation. However, it is important to quantify the interaction between genetics, morphology and signalling behaviour to understand the extent to which signal diversification might cause reproductive isolation. We have demonstrated that the display behaviour of the Jacky dragon is quite labile across its range, and we suggest that behavioural plasticity might be the cause of this interesting variation.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (Macquarie University's Animal Ethics Committee No. ARA 2010/034) or practice at which the studies were conducted.

References

Barnard P (1991) Ornament and body size variation and their measurement in natural populations. Biol J Linn Soc 42:379–388

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-7, http://CRAN.R-project.org/package=lme4. Accessed 20 October 2014

Bensch S, Hasselquist D, Nielsen B, Hansson B (1998) Higher fitness for philopatric than for immigrant males in a semi-isolated population of great reed warblers. Evolution 52:877–883

Birkhead TR (1978) Behavioural adaptations to high density nesting in the common guillemot *Uria aalge*. Anim Behav 26:321–324

Bloch N, Irschick DJ (2006) An analysis of inter-population divergence in visual display behavior of the green anole lizard (*Anolis carolinensis*). Ethology 112:370–378

Blumstein DT, Daniel JC (2007) Quantifying behavior the JWatcher way. Sinauer Associates, Massachusetts

Carpenter CC, Ferguson GW (1977) Variation and evolution of stereotyped behavior in reptiles. In: Gans C, Tinkle D (eds) Biology of the reptilia: ecology and behavior A, vol 7. Academic Press, New York, pp 335–554

Carpenter CC, Badham JA, Kimble B (1970) Behavior patterns of three species of *Amphibolurus* (Agamidae). Copeia 1970:497–505

Cogger HG (2000) Reptiles and amphibians of Australia, 6th edn. Reed New Holland, Sydney

Dunbrack R, Clarke L (2003) Escalated aggression in interpopulation brook trout dyads: evidence for behavioural divergence. Can J Zool 81:911–915

Endler JA (1987) Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). Anim Behav 35:1376–1385

Endler JA, Westcott DA, Madden JR, Robson T (2005) Animal visual systems and the evolution of color patterns: sensory processing illuminates signal evolution. Evolution 59:1795–1818

Ferguson GW (1971) Variation and evolution of the push-up displays of the side-blotched lizard genus *Uta* (Iguanidae). Syst Zool 20:79–101

Fleishman LJ (1992) The influence of the sensory system and the environment on motion patterns in the visual displays of anoline lizards and other vertebrates. Am Nat 139:S36–S61

Foster SA (1999) The geography of behaviour: an evolutionary perspective. Trends Ecol Evol 14:190–195

Harlow PS, Taylor JE (2000) Reproductive ecology of the jacky dragon (*Amphibolurus muricatus*): an agamid lizard with temperature-dependent sex determination. Austral Ecol 25:640–652

Hill GE (1994) Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. Behav Ecol 5:64–73

Irwin DE, Thimgan MP, Irwin JH (2008) Call divergence is correlated with geographic and genetic distance in greenish warblers (*Phylloscopus trochiloides*): a strong role for stochasticity in signal evolution? J Evol Biol 21:435–448

Jenssen TA (1971) Display analysis of *Anolis nebulosus* (Sauria, Iguanidae). Copeia 1971:197–209

Jenssen TA (1977) Evolution of anoline lizard display behavior. Am Zool 17:203–215

Leal M, Fleishman LJ (2004) Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. Am Nat 163:26–39

Lovern MB, Jenssen TA, Orrell KS, Tuchak T (1999) Comparisons of temporal display structure across contexts and populations in male *Anolis carolinensis*: signal stability or lability? Herpetologica 55: 222–234

Martins EP, Bissell AN, Morgan KK (1998) Population differences in a lizard communicative display: evidence for rapid change in structure and function. Anim Behav 56:1113–1119

Ord TJ, Evans CS (2003) Display rate and opponent assessment in the Jacky dragon (*Amphibolurus muricatus*): an experimental analysis. Behaviour 140:1495–1508

Ord TJ, Blumstein DT, Evans CS (2002) Ecology and signal evolution in lizards. Biol J Linn Soc 77:127–148

Ord TJ, Peters RA, Clucas B, Stamps JA (2007) Lizards speed up visual displays in noisy motion habitats. Proc R Soc Lond B 274:1057–1062

Pepper M, Barquero MD, Whiting MJ, Keogh JS (2014) A multi-locus molecular phylogeny for Australia's iconic Jacky Dragon (Agamidae: *Amphibolurus muricatus*): phylogeographic structure along the Great Dividing Range of south-eastern Australia. Mol Phylogenet Evol 71:149–156

Persons MH, Fleishman LJ, Frye MA, Stimphil ME (1999) Sensory response patterns and the evolution of visual signal design in anoline lizards. J Comp Physiol A 184:585–607

Peters RA (2008) Environmental motion delays the detection of movement-based signals. Biol Lett 4:2–5

Peters RA, Evans CS (2003) Introductory tail-flick of the Jacky dragon visual display: signal efficacy depends upon duration. J Exp Biol 206:4293–4307

Peters RA, Ord TJ (2003) Display response of the Jacky Dragon, *Amphibolurus muricatus* (Lacertilia: Agamidae), to intruders: a semi-Markovian process. Austral Ecol 28:499–506

Peters RA, Hemmi JM, Zeil J (2007) Signaling against the wind: modifying motion-signal structure in response to increased noise. Curr Biol 17:1231–1234

Podos J (2001) Correlated evolution of morphology and vocal signal structure in Darwin's finches. Nature 409:185–188

Snell-Rood EC (2013) An overview of the evolutionary causes and consequences of behavioural plasticity. Anim Behav 85:1004–1011

Steinberg DS, Losos JB, Schoener TW, Spiller DA, Kolbe JJ, Leal M (2014) Predation-associated modulation of movement-based signals by a Bahamian lizard. Proc Natl Acad Sci U S A 111:9187–9192

Stuart-Fox DM, Moussalli A, Marshall NJ, Owens IPF (2003) Conspicuous males suffer higher predation risk: visual modeling and experimental evidence from lizards. Anim Behav 66:541–550

Stuart-Fox D, Moussalli A, Whiting MJ (2007) Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. Am Nat 170:916–930

Uy JAC, Borgia G (2000) Sexual selection drives rapid divergence in bowerbird display traits. Evolution 54:273–278

Watt MJ, Joss JMP (2003) Structure and function of visual displays produced by male Jacky Dragons, *Amphibolurus muricatus*, during social interactions. Brain Behav Evol 61:172–183

White J (1790) Journal of a voyage to New South Wales, with sixty-five plates of non descript animals, birds, lizards, serpents, curious cones of trees and other natural productions. J. Debrett, London

Wiens JJ (2000) Decoupled evolution of display morphology and display behaviour in phrynosomatid lizards. Biol J Linn Soc 70:597–612

Young JR, Hupp JW, Bradbury JW, Braun CE (1994) Phenotypic divergence of secondary sexual traits among sage grouse, *Centrocercus urophasianus*, populations. Anim Behav 47:1353–1362