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Sexual selection predicts brain structure in dragon lizards

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Abstract

Phenotypic traits such as ornaments and armaments are generally shaped by sexual selection, which often favours larger and more elaborate males compared to females. But can sexual selection also influence the brain? Previous studies in vertebrates report contradictory results with no consistent pattern between variation in brain structure and the strength of sexual selection. We hypothesize that sexual selection will act in a consistent way on two vertebrate brain regions that directly regulate sexual behaviour: the medial preoptic nucleus (MPON) and the ventromedial hypothalamic nucleus (VMN). The MPON regulates male reproductive behaviour whereas the VMN regulates female reproductive behaviour and is also involved in male aggression. To test our hypothesis, we used high-resolution magnetic resonance imaging combined with traditional histology of brains in 14 dragon lizard species of the genus *Ctenophorus* that vary in the strength of precopulatory sexual selection. Males belonging to species that experience greater sexual selection had a larger MPON and a smaller VMN. Conversely, females did not show any patterns of variation in these brain regions. As the volumes of both these regions also correlated with brain volume (BV) in our models, we tested whether they show the same pattern of evolution in response to changes in BV and found that the do. Therefore, we show that the primary brain nuclei underlying reproductive behaviour in vertebrates can evolve in a mosaic fashion, differently between males and females, likely in response to sexual selection, and that these same regions are simultaneously evolving in concert in relation to overall brain size.

Introduction

Sexual selection favours the evolution of traits that promote success in competition for mates or gametes (Andersson, 1994). Classical examples of premating sexual selection mechanisms include behavioural traits such as male contest competition and mate choice. Underlying all behaviour is brain function, and therefore, brains should experience selection pressures imposed by mate choice and sexual selection more generally (Cahill & Aswad, 2015). Empirical evidence for sexual selection acting on brains is rare, but where it exists no overall pattern emerges. Different studies across a range of vertebrate species have found evidence that species under strong sexual selection either have smaller (Garamszegi *et al.*, 2005; Pitnick *et al.*, 2006; Fitzpatrick *et al.*, 2012; García-Peña *et al.*, 2013) or larger (Madden, 2001; Garamszegi, 2004) brains, or brain size does not vary with the strength of sexual selection (Iwaniuk, 2001; Schillaci, 2006; Guay & Iwaniuk, 2008; Lemaitre *et al.*, 2009; Gonzalez-Voyer & Kolm, 2010). Studies that find positive correlations

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between sexual selection and brain size argue that sexual selection increases cognitive demands resulting in larger brains (Sherry *et al.*, 1992; Lindenfors *et al.*, 2007; García-Peña *et al.*, 2013). Studies that find negative correlations between sexual selection and brain size argue that sexual selection increases energetic demands, resulting in a trade-off between allocating energy to brain tissue and reproduction (Pitnick *et al.*, 2006; García-Peña *et al.*, 2013). These hypotheses are not mutually exclusive, nor do they offer criteria that preclude competing explanations, and therefore, no unifying theory exists.

Instead of evolving in concert as a single unit, it is possible that the various subdivisions of the brain are evolving independently of each other as a mosaic in response to sexual selection (Stanyon & Bigoni, 2014; Joel & Fausto-Sterling, 2016). However, when the subdivisions are analysed separately, still no consistent pattern has emerged. For example, a study in cichlid fishes found that the cerebellum is one of the only neural subdivisions under sexual selection (Gonzalez-Voyer & Kolm, 2010), whereas a study in primates found that it is one of the only subdivisions not influenced by sexual selection (Lindenfors *et al.*, 2007). Although these contrasting results may be explained by phylogenetic distance, conflicting findings sometimes occur in the same group. In primates, three studies found three different relationships between neocortical volume and sexual selection (Pawłowski *et al.*, 1998; Lindenfors *et al.*, 2007; Schillaci, 2008).

Ultimately, there may not be any consistent relationship between sexual selection and the volume of the brain or its major subdivisions (Dechmann & Safi, 2009; García-Peña *et al.*, 2013) because the complex and diverse functions of the brain lead to selection pressures in different directions for different brain regions (Iwaniuk, 2001; Healy & Rowe, 2007, 2013; Cahill & Aswad, 2015). A better understanding of the relationship between the brain and sexual selection will come from targeting specific brain nuclei with well-characterized functions involved in reproduction (Ball *et al.*, 2014) and by selecting a model system of closely related species that differ in key traits and which have a well-resolved phylogeny to control for relatedness (Dechmann & Safi, 2009). These approaches have already provided important and novel insights into the evolution and the neural underpinnings of vocal learning (Jacobs, 1996; Jarvis *et al.*, 2005; Pfenning *et al.*, 2014).

Across all vertebrates, the brain regions that regulate reproductive behaviour are functionally conserved as part of the broader 'social behaviour network' (Goodson, 2005; O'Connell & Hofmann, 2011). Detailed analysis on structure and function shows that the medial preoptic nucleus (MPON) is the key brain region regulating male reproductive behaviour (Hart & Leedy, 1985; Nieuwenhuys *et al.*, 1998; Balthazart & Ball, 2007; O'Connell & Hofmann, 2011; Numan, 2014), whereas the ventromedial hypothalamic nucleus (VMN) is the key brain region regulating female reproductive behaviour (Cooke *et al.*, 1998; Nieuwenhuys *et al.*, 1998; O'Connell & Hofmann, 2011; Numan, 2014). Variation in the volume of these regions is associated with variation in sexual activity experimentally (Anderson *et al.*, 1986; Wade *et al.*, 1993; Houtsmuller *et al.*, 1994; Crews *et al.*, 1998; Roselli *et al.*, 2004) and in nature (Shapiro *et al.*, 1991; Crews *et al.*, 1993; Beck *et al.*, 2008; Beck & Wade, 2009; Wade, 2011).

The sex-specific functions of these nuclei suggest that they are likely to be sexually dimorphic. In some taxa, this is true. For example, the MPON is larger in males (male-biased sexual dimorphism) of Japanese quail, *Anolis* lizards, rats and humans (Gorski *et al.*, 1978; Swaab & Fliers, 1985; Viglietti-Panzica *et al.*, 1986; Beck *et al.*, 2008). However, the MPON is not always sexually dimorphic. It is monomorphic in species such as leopard geckos, mice and macaque monkeys (Young, 1982; Ayoub *et al.*, 1983; Coomber *et al.*, 1997). Volumetric data on the VMN are rare, but the VMN is larger in females (female-biased sexual dimorphism) in the lizard *Cnemidophorus inornatus*, monomorphic in several other reptile species and larger in males in rats and the lizard *Anolis carolinensis* (Matsumoto & Arai, 1983; Crews *et al.*, 1990; Godwin *et al.*, 1997; Beck & Wade, 2009).

These contrasting examples show that sexual dimorphism in MPON and VMN volume is not consistent across species, and we hypothesize that sexual dimorphism in these brain regions is related to the strength of sexual selection. In a pairwise comparison between two sister species of voles (*Microtus*) in which one species is monogamous and the other is promiscuous, the MPON was sexually dimorphic only in the species under strong sexual selection (Shapiro *et al.*, 1991). In a similar comparison between two fence lizards (*Sceloporus*), only the species under strong sexual selection had sexually dimorphic aromatase-expressing cell counts in the MPON and VMN (Hews *et al.*, 2012). Based on these studies, we propose that strong sexual selection increases sexual dimorphism in brain regions that control sexual activity, as these regions have a direct impact on reproductive success, the ultimate target of sexual selection.

To test our hypothesis, we selected closely related dragon lizard species in the Australian genus *Ctenophorus* (Hamilton *et al.*, 2015), which vary in the apparent strength of sexual selection they experience, and for which there is a robust phylogeny (Chen *et al.*, 2012). We used three standard indices of precopulatory sexual selection: sexual dichromatism (SDC), body size sexual dimorphism (BSSD) and head size sexual dimorphism (HSSD). These indices are widely used as indicators of the strength of sexual selection in comparative studies (Andersson, 1994; Stuart-Fox & Ord, 2004; Fairbairn *et al.*, 2008; Chen *et al.*, 2012) and have been empirically shown to be associated with sexual selection in

lizards (Cox *et al.*, 2003; Sullivan & Kwiatkowski, 2007; Fairbairn *et al.*, 2008; Cox & Calsbeek, 2009). It is possible that these three different indices represent different components of sexual selection (Kraaijeveld *et al.*, 2011) and that variation in each measure of dimorphism represents the strength of the particular component of sexual selection. Therefore, we have used all three indices of sexual selection at our disposal to try and determine whether sexual selection is influencing the evolution of the brain.

In *Ctenophorus*, some species show marked SDC, with conspicuously coloured males and cryptically coloured females, whereas in other species, both males and females are cryptically coloured (Stuart-fox *et al.*, 2008; Chen *et al.*, 2012). Similarly, both HSSD and BSSD show great variation across *Ctenophorus* species (Chen *et al.*, 2012). According to Chen *et al.* (2012), all three indices of sexual selection are more variable across *Ctenophorus* than any other group of Australian agamids. Both SDC and BSSD are important factors for intraspecific communication in this group, including male–male competition and mate choice (LeBas & Marshall, 2000; Stuart-Fox & Ord, 2004; Healey *et al.*, 2007; Johnston, 2011; Osborne *et al.*, 2012; Umbers *et al.*, 2012; Yewers *et al.*, 2016). The extent of the variation in these characters between *Ctenophorus* species, coupled with their importance for intraspecific signalling in this group, made *Ctenophorus* an ideal system in which to test our hypothesis.

We predicted that species under strong sexual selection would have male-biased MPON sexual dimorphism and female-biased VMN sexual dimorphism, but no difference in brain volume (BV) dimorphism. This prediction is based on the mosaic hypothesis of brain evolution, where individual brain regions respond independently to specific selection pressures (Barton & Harvey, 2000). The alternate hypothesis, concerted brain evolution, proposes that because brain regions are anatomically and developmentally linked, changes in brain regions will be correlated (Finlay & Darlington, 1995). These modes are not mutually exclusive, and the current view is that both modes are occurring simultaneously in response to different selection pressures (Striedter, 2005; Gutiérrez-Ibáñez *et al.*, 2014; Corfield *et al.*, 2015). Therefore, we also tested the hypothesis that the MPON and the VMN evolve in concert relative to the evolution of the volume of the entire brain. We also measured the volumes of two unrelated nuclei, the dorsomedial thalamic (DMT) and dorsolateral hypothalamic (DLH) nuclei, as controls.

Materials and methods

Specimen acquisition

We collected 296 lizards from 14 *Ctenophorus* species during the early part of the breeding season (September and October). Following capture, lizards were transported to the Australian National University in Canberra, Australia, where they were maintained in outdoor enclosures with *ad libitum* access to food (wild insects) and water, and their diet was supplemented twice weekly with domestic crickets. The Australian National University's Animal Experimental Ethics Committee approved all research under protocol number A201149.

Sexual dichromatism

All lizard reflectance spectra were recorded within 6 weeks of capture. To ensure the most realistic spectra, while awaiting spectral processing, lizards were housed in a terrarium with a basking light so they could attain their preferred active daytime temperature. Lizards were handled with gloved hands, a common method of restraint during spectral measurement. *Ctenophorus* do not dynamically change colour and therefore do not change colour over the short period of time they are restrained. Spectra were sampled during the day with an Ocean Optics Jaz spectrometer, PX-2 light source, and bifurcated fibre optic probe. A black anodized aluminium probe cover cut at 45° to avoid specular reflectance held the end of the fibre optic probe at a distance of 5 mm from the lizard's skin. Spectral measurements were taken at points on a grid across the dorsal and lateral surfaces of each lizard

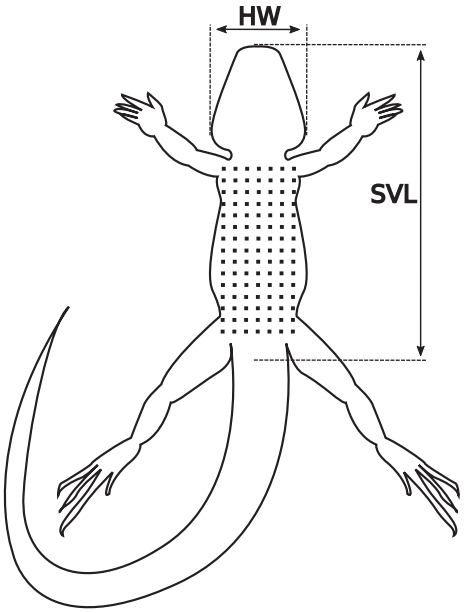

Fig. 1 Schematic of the dorsal surface of the lizard showing the measurements we took for each dragon. Spectral measurements were taken at each point in the grid on the body of the lizard. Grid orientation was consistent across all species; however, grid dimensions and spacing varied between species according to pattern complexity. The grid shown here has the dimensions used for the bicycle dragon (*Ctenophorus cristatus*). HW, head width; SVL, snout–vent length.

(Fig. 1). Spacing between sample points was constant within species but ranged from 3.5 to 7.0 mm among species, with smaller spacing for finer grained colour patterns (Endler, 2012). One axis of the grid was aligned with the body axis, and sampling locations were consistent between individuals (but not between species) with respect to landmarks such as the head and limbs. Between 50 and 130 spectral measurements were taken per lizard; the number of sampling points varied between species but was consistent within species (Endler, 2012).

The degree of SDC was estimated from the reflectance spectra as follows. For every reflectance spectrum, we smoothed (20 points or about 7 nm window) and interpolated to 2-nm intervals using the MATLAB functions smooth and pchip. We included only spectra between 300 and 700 nm, which comfortably incorporates the visible spectrum of lizards in general and the tawny dragon (*Ctenophorus decresii*) in particular (Endler & Mielke, 2005; Yewers *et al.*, 2015). Separately for each species and sex, we calculated the standard deviation of reflectance at each wavelength over all points. This resulted in a SDC variance spectrum for males and females for each species. We then calculated the relative difference between sexes in the standard deviation of the reflectance at each wavelength. To calculate this measure of SDC, we used the formula $d = 2(\text{M-F})/(\text{M+F})$, where M = male value, and F = female value; in this case, 'value' refers to the standard deviation of reflectance. For each species, this resulted in 201 values of d, each value representing the standardized difference between sexes in the standard deviation of the reflectance for one sampled wavelength. We used the maximum of d as a measure of sexual dichromatism. SDC of reflectance is a measure of within-pattern contrast because the more variable the reflectance is among sample points, the greater the visual contrast. If $d > 0$, males have more visual contrast, and if $d < 0$, females have more contrast.

Body and head size sexual dimorphism

For each lizard, we measured snout–vent length (SVL) to the nearest millimetre using a transparent ruler and head width to the nearest 0.01 mm using digital callipers. We then used these measures to calculate the sexual dimorphism measure d for both traits. In these cases, the male and female values were mean SVL and mean head width for each species. Positive values indicate male-biased dimorphism, whereas negative values indicate female-biased dimorphism (Table 1).

Table 1 Sexual dimorphism in *Ctenophorus*. Values are d for sexual dichromatism (SDC), head size sexual dimorphism (HSSD) and body size sexual dimorphism (BSSD). See methods for the formula for d.

Ctenophorus species		Sexual selection index		
Common name	Scientific name	SDC	HSSD	BSSD
Central Ring-tailed Dragon	*C. caudicinctus slateri*	0.479	0.054	0.017
Bicycle Dragon	*C. cristatus*	0.291	0.010	0.000
Tawny Dragon	*C. decresii*	0.705	0.054	0.021
Peninsula Dragon	*C. fionni*	0.894	0.069	0.020
Mallee Military Dragon	*C. fordi*	0.279	−0.001	−0.001
Gibber Dragon	*C. gibba*	0.439	0.011	0.002
Central Military Dragon	*C. isolepis gularis*	1.019	0.008	0.001
Central Netted Dragon	*C. nuchalis*	0.168	0.021	0.021
Ornate Dragon	*C. ornatus*	0.242	0.054	0.025
Painted Dragon	*C. pictus*	0.927	0.031	0.004
Rusty Dragon	*C. rufescens*	0.230	0.031	0.008
Claypan Dragon	*C. salinarum*	0.420	0.035	0.007
Ochre Dragon	*C. tjantjalka*	0.891	0.068	0.042
Red-backed Dragon	*C. vadnappa*	1.053	0.075	0.042

Calculating whole-brain volume using magnetic resonance imaging

Before the end of the breeding season (December), each lizard was perfused as described in Hoops (2015). Magnevist (gadopentetate dimeglumine, Bayer) was added to the fixative perfusate and storage buffer at a concentration of 0.01% to maximize image contrast in magnetic resonance imaging. Whole-brain images were acquired using a Bruker Avance 11.74 Tesla wide-bore spectrometer (Ettlingen, Germany) with a micro-2.5 imaging probe capable of generating magnetic gradients of 1.50 T m^{-1}. Parameters used in the scans were optimized for grey-white matter contrast in the presence of Magnevist. We used a gradient-echo (T_2*-weighted) 3D fast gradient-echo sequence (FLASH), with a repetition time = 40 ms, echo time = 8 ms field-of-view = 11 × 11 × 16 mm and matrix size = 110 × 110 × 160, producing an image with 100-μm^3 isotropic voxels.

To ensure consistent measures of brain morphometry, all images were first manually masked using the 3D analysis software Avizo (FEI, Hillsoboro, Oregon) such that consistent coverage of brain structures and nerve endings was achieved. The manually masked areas were then set to the background value such that they were not included in subsequent calculations. BV was then calculated in the MINC toolkit via a histogram cut-off value for image and background using a minimum error thresholding algorithm (Kittler & Illingworth, 1986).

Calculating brain region volumes using histology

Brains were sectioned in the coronal (anterior–posterior) plane and stained using SYBR-green (Life Technologies Australia, Melbourne, Australia). Images were captured using an Olympus BX63 microscope, a XM10 digital camera and the imaging-stitching function of the Olympus CellSens software package (Fig. 2). The areas of the MPON, VMN, DMT and DLH were measured,

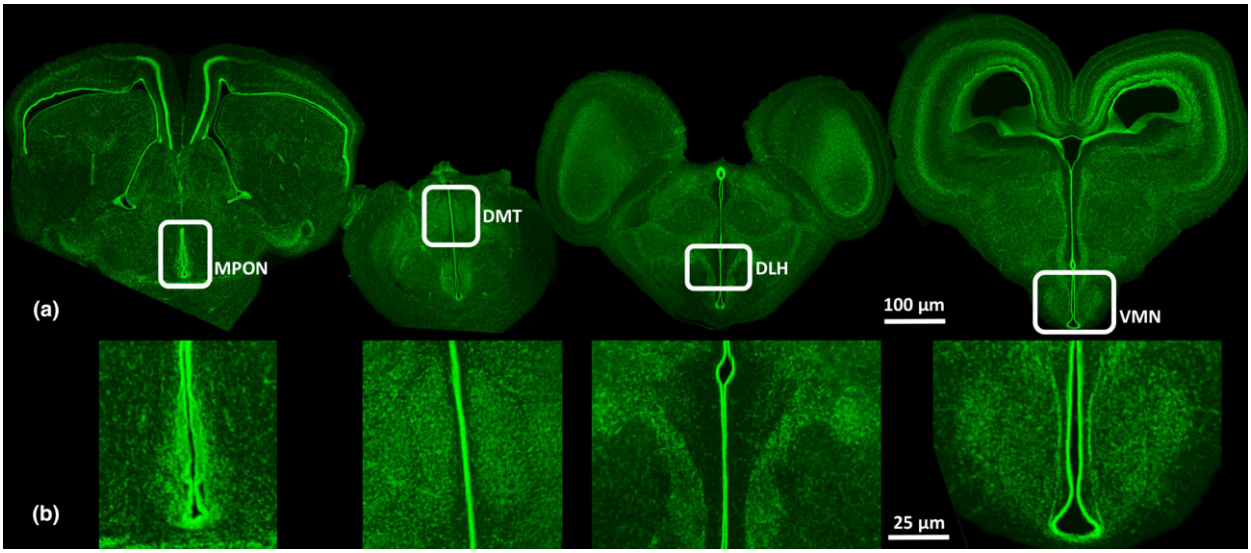

Fig. 2 Fluorescent-stained coronal sections depicting the locations of the MPON, VMN, DMT and DLH in the brain of a *Ctenophorus* dragon. Coronal sections are anterior–posterior left to right. (a) depicts an entire coronal section with the brain region of interest circled. (b) is an enlargement of the region of interest from the image in (a). DLH, dorsolateral hypothalamic nucleus; DMT, dorsomedial thalamic nucleus; MPON, medial preoptic nucleus; VMN, ventromedial hypothalamic nucleus

blind to sex, by a single person using the Count & Measure CellSens module. Volumes were calculated by multiplying the areas by the slice thickness (Kabelik *et al.*, 2006). As there is no atlas available for a *Ctenophorus* brain, or the brain of any agamid, to identify and delineate our regions of interest we consulted available lizard brain atlases (Northcutt, 1967; Butler & Northcutt, 1973; Cruce, 1974; Greenberg, 1982; Smeets *et al.*, 1986; Del Corral *et al.*, 1990; Medina *et al.*, 1992, 1993; ten Donkelaar, 1998) and neuroanatomists and developed our own atlas of the tawny dragon *(C. decresii)* brain (D. Hoops, E. Desfilis, J.F.P. Ullmann, A.J. Janke, T. Stait-Gardner, W.S. Price, L. Medina, M.J. Whiting and J.S. Keogh, in preparation).

Statistical analysis

Although SDC, HSSD and BSSD may represent different components of sexual selection, these components are not easily defined or separated, nor are they necessarily independent (Kraaijeveld *et al.*, 2011). To obtain an indication as to whether our three indices are interdependent, we calculated phylogenetic generalized least squares (PGLS) using the R (R Core Team, 2014) package geiger (Harmon *et al.*, 2008) to determine whether our three indices of sexual selection are correlated independent of phylogeny.

We analysed 287 brains to determine the relationship between brain region volume and the indices of sexual selection; however, tissue damage to one or more brain regions in some specimens meant that we could not measure every brain region in every brain (Electronic Supplementary Materials, ESM 1). All volumes were $\log_{10}$-transformed prior to analysis. We fitted phylogenetic linear models separately for each brain region. Phylogenetic information, including relationships among species and branch lengths, was incorporated into our analysis with a robust time-calibrated molecular phylogeny (Chen *et al.*, 2012). We used the Brownian motion model of trait evolution (Pagel, 1999) in the R package *phylolm* (Tung Ho & Ane, 2014). As it was not possible to have separate values for each sex in a phylogenetically controlled model, we calculated the sexual dimorphism measure *d* for each brain region. We generated three models per brain region: a model with *d* as the response variable, one with male volume as the response variable, and one with female volume as the response variable. As independent factors we entered into each model our sexual selection indices, excluding those that were shown to be significantly correlated to other indices according to PGLS. A size correction variable was included in each model: snout–vent length for models examining BV, and BV for models examining MPON, VMN, DMT and DLH volumes.

We used the natural model averaging (Symonds & Moussalli, 2010; Grueber *et al.*, 2011) method of the R package *MuMIn* (Barton, 2014) to generate models of all possible combinations of our independent factors and to calculate model-averaged estimates from models where the corrected Akaike information criterion (AICc) (Hurvich & Tsai, 1989) was within two of the lowest AICc (ΔAICc ≤ 2), ΔAICc ≤ 4, ΔAICc ≤ 6 and all models within the 95% confidence interval of summed weights (Symonds & Moussalli, 2010; Grueber *et al.*, 2011). Because of the low number of models in the ΔAICc ≤ 2 model set and the high number of models in

the latter two sets, here we present the ΔAICc ≤ 4 average model. For our data set, similar results were obtained when using different AICc cut-offs (ESM 2). We report relative importance (RI), a measure specific to model averaging that takes into account both the number of models in which a factor appears and the quality of those models (based on their AICc) to estimate the importance of a factor in predicting variation in the responding variable (Symonds & Moussalli, 2010). In addition, factors that had an important effect on a response variable have slope estimates with 95% confidence intervals (CI) that do not include zero (Grueber *et al.*, 2011).

Subsequent to our main analysis, we conducted a *post hoc* analysis to test the relationships between brain region volume and BV. We used a phylogenetically corrected standard major axis regression (PSMAR) in the R package phytools (Revell, 2011) to determine whether the MPON, VMN, DMT and DLH all show similar relationships to BV and therefore are evolving in concert (Powell & Leal, 2012). Standard major axis regressions test whether the slope of a regression line (b) is significantly different from 1. Because of this, in both the case where $b = 1$ and the case where there is no significant relationship between two variables, $P > 0.05$. For this reason, the PSMAR should be used as a *post hoc* test once a significant relationship has been established (Smith, 2009; Revell, 2011). Therefore, we only used PSMAR to test whether $b = 1$ (null hypothesis) or $b \neq 1$ (alternate hypothesis) for brain regions for which BV was a significant predictor in the model-averaged estimates. All data used in this study are available as supplementary materials to this article (ESM3).

Results

Test for relationships between indices of sexual selection

SDC was not related to either BSSD or HSSD; however, BSSD and HSSD were significantly related (Table 2). Therefore, HSSD was excluded from further analyses to avoid multicollinearity.

Table 2 Phylogenetic generalized least squares. We tested whether our three indices of sexual selection were correlated with each other to avoid multicollinearity in further analyses. Factors in bold are significantly correlated.

Factors	Slope	SE	r^2	$F_{1,12}$	P
SDC, BSSD	1.715	1.377	0.041	1.552	0.237
SDC, HSSD	2.334	1.335	0.137	3.057	0.106
BSSD, HSSD	**0.749**	**0.131**	**0.708**	**32.55**	**9.8×10^{-5}**

BSSD, body size sexual dimorphism; HSSD, head size sexual dimorphism; SDC, sexual dichromatism; SE, standard error.

SDC predicts MPON and VMN volume

SDC was an important predictor of both MPON dimorphism and VMN dimorphism but was positively correlated with MPON dimorphism and negatively correlated with VMN dimorphism (Table 3; Fig. 3). As male-biased dimorphism is positive and female-biased dimorphism is negative, this indicates that, as SDC becomes more pronounced, the MPON becomes larger in males and the VMN larger in females. SDC was not an important predictor of brain (Fig. 4a), DMT or DLH dimorphism (Table 3; Fig. 3). Moreover, BSSD was not important for predicting the volume of any brain region (Table 3; Fig. 3).

To determine the extent to which these differences in dimorphism involved variation in males and females, we examined brain, MPON, VMN, DMT and DLH volumes separately for each sex. No index of sexual selection was important for predicting brain (Fig. 4b), DMT or DLH volume in males or any volume in females (Table 3; Fig. 5). However, SDC had strong predictive value for both the MPON and the VMN in males. As species became more sexually dichromatic, male MPON volume increased, whereas VMN volume decreased (Table 3; Fig. 5). Therefore, we conclude that the MPON and the VMN are evolving independently within the brain, likely in response to sexual selection.

All brain regions show concerted brain evolution with respect to BV

We tested each brain region that was significantly correlated with BV in the averaged linear model estimates (Table 3) for concerted evolution with respect to BV. We found that all brain regions evolved in proportion to BV; we could not reject the null hypothesis of $b = 1$ in any test (Table 4). Therefore, we conclude that the MPON (in males), VMN, DMT and DLH are evolving in concert with respect to BV.

Discussion

As hypothesized, we did not find any differences in whole-brain sexual dimorphism with any index of sexual selection. However, we did find variation in specific brain nuclei correlated with sexual selection. Our results support our hypothesis that sexual dimorphism in the brain regions responsible for reproductive behaviour increases with the strength of sexual selection. Males from more sexually dichromatic species had larger medial preoptic nuclei (MPON). Although we predicted that females would have larger ventromedial hypothalamic nuclei (VMN), we only found this relationship because there appeared to be selection for smaller VMNs in males as SDC increased. Sexual dimorphism was not related to the volume of either control region.

		Dimorphism			Males			Females		
Region	Factor	Estimate	SE	RI	Estimate	SE	RI	Estimate	SE	RI
Brain	Intercept	−0.475	0.060	–	**2.195**	**0.385**	–	**2.690**	**0.333**	–
	SVL	0.046	0.028	0.39	**1.578**	**0.200**	**1.00**	**1.312**	**0.172**	**1.00**
	SDC	−0.001	0.007	0.07	0.045	0.048	0.18	0.072	0.040	0.36
	BSSD	0.251	0.154	0.39	–	–	–	1.307	0.965	0.18
MPON	Intercept	−0.058	0.125	–	**4.419**	**1.502**	–	**6.466**	**1.390**	–
	BV	0.043	0.025	0.21	**0.605**	**0.212**	**1.00**	0.338	0.320	0.19
	SDC	**0.045**	**0.013**	**1.00**	**0.255**	**0.089**	**0.86**	−0.096	0.111	0.16
	BSSD	−0.302	0.321	0.15	–	–	–	0.664	2.640	0.11
VMN	Intercept	0.011	0.026	–	**4.515**	**0.736**	–	**3.138**	**0.680**	–
	BV	−0.008	0.014	0.09	**0.632**	**0.140**	–	**0.887**	**0.131**	**1.00**
	SDC	***−0.015***	***0.005***	***0.91***	***−0.202***	***0.060***	–	−0.078	0.042	0.46
	BSSD	0.183	0.114	0.30	0.848	1.376	–	–	–	–
DMT	Intercept	−0.038	0.075	–	**3.352**	**0.552**	–	**3.450**	**0.898**	–
	BV	0.018	0.017	0.21	**0.827**	**0.106**	**1.00**	**0.806**	**0.173**	**1.00**
	SDC	0.004	0.006	0.14	−0.034	0.043	0.14	−0.074	0.058	0.24
	BSSD	−0.027	0.149	0.11	−0.701	0.995	0.13	0.471	1.429	0.09
DLH	Intercept	−0.025	0.067	–	**3.310**	**0.864**	–	**4.000**	**1.485**	–
	BV	0.177	0.020	0.14	**0.747**	**0.165**	**1.00**	**0.663**	**0.240**	**0.88**
	SDC	0.007	0.007	0.16	−0.077	0.066	0.20	−0.143	0.082	0.40
	BSSD	0.217	0.167	0.23	1.307	0.965	0.13	−1.956	1.950	0.11

Table 3 Averaged phylogenetic linear model estimates. Factors in bold are directly correlated with the response variable; their confidence intervals (CI) lie above zero. Factors in bold italics are inversely correlated with the response variable; their CI lie below zero. Factors which are not highlighted are not significantly correlated with the response variable; their CI overlap zero. Models examining dimorphism use the measure *d* of each brain region as the dependent variable, whereas models of males and females separately use brain region volume as the dependent variable. RI values are absent from the male VMN model-averaged estimate as there is only one model in the set $\Delta AICc \leq 4$.

AICc, Akaike information criterion; BSSD, body size sexual dimorphism; BV, brain volume; DLH, dorsolateral hypothalamic nucleus; DMT, dorsomedial thalamic nucleus; MPON, medial preoptic nucleus; SDC, sexual dichromatism; RI, relative importance; SE, standard error; SVL, snout–vent length; VMN, ventromedial hypothalamic nucleus.

The MPON was larger in males from species that we estimated are under relatively stronger sexual selection. The MPON is the key brain region necessary for consummatory reproductive behaviour (e.g. courtship, mounting, erection, ejaculation) in male vertebrates (Hart & Leedy, 1985; Numan, 2014). The MPON also facilitates appetitive male reproductive behaviour (sexual motivation or mate seeking) (Hart & Leedy, 1985; Pfaus & Phillips, 1991; Hurtazo *et al.*, 2008; Numan, 2014). In male vertebrates, larger MPONs are associated with increases in reproductive behaviour (Anderson *et al.*, 1986). The mechanisms underlying this correlation remain unclear, and further experimental testing of the nature of the relationship between MPON volume and sexual behaviour is an important future direction of this study.

Although we found support for our hypothesis of increasing female-biased VMN sexual dimorphism under stronger sexual selection, the underlying pattern was unexpected: there was a reduction in male VMN volume with increasing SDC. In male rodents, and likely male lizards, a subregion of the VMN is involved in regulating aggression (Lin *et al.*, 2012; Yang *et al.*, 2013; de Boer *et al.*, 2015; Falkner *et al.*, 2016)(Kabelik *et al.*, 2008). Furthermore, in many vertebrates, including lizards, intrasexual aggression is associated with territory maintenance and establishing dominance, behaviour that influences reproductive success in many mating systems (Husak *et al.*, 2009). Therefore, aggression under certain circumstances may be positively sexually selected and we might predict that the VMN would be larger in more sexually selected species (Andersson, 1994). However, our findings suggest the opposite: species under relatively strong sexual selection have female-biased VMN dimorphism. Previous findings in lizards show that VMN dimorphism may be biased in either direction: VMN volume may be larger in males, monomorphic, or larger in females (Crews *et al.*, 1990; Godwin *et al.*, 1997; Beck & Wade, 2009). This variation in VMN volume in males may be related to the evolutionary costs associated with increased aggression. For example, male leopard geckos incubated at abnormally high temperatures have larger VMNs (Coomber *et al.*, 1997), are more likely to respond to females with aggressive behaviour instead of reproductive behaviour (Flores *et al.*, 1994), and consequently experience a fitness cost. It is possible that a reduction in VMN volume may ameliorate the costs of aggressive behaviour in circumstances where it would be disadvantageous. The effect size of this unexpected result is relatively small compared to the size of the effect in MPON volume. We suggest that this may be because the VMN is not directly associated with sexual behaviour in males and therefore is unlikely to be directly influenced by sexual selection. We hypothesize that VMN volume is

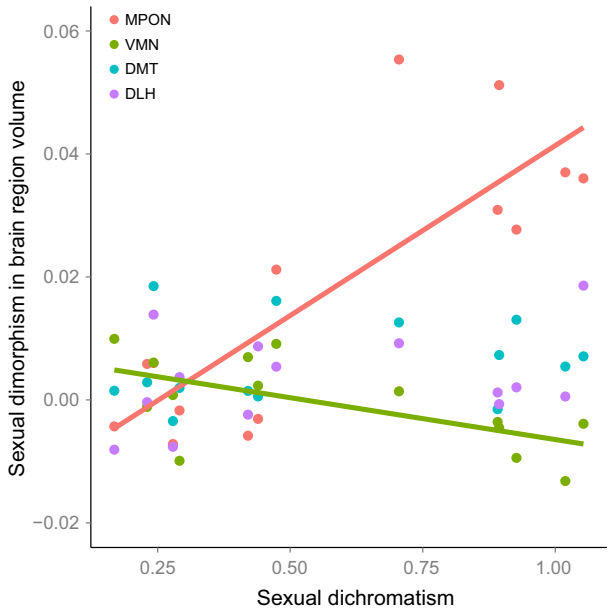

Fig. 3 Sexual dimorphism in the MPON and VMN correlates with sexual dichromatism, whereas sexual dimorphism in the DMT and DLH does not. Points are sexual dimorphism values (*d*) for each species. Values above zero represent male-biased dimorphism, and values below zero represent female-biased dimorphism. Regression lines are shown for relationships that have a nonzero slope. DLH, dorsolateral hypothalamic nucleus; DMT, dorsomedial thalamic nucleus; MPON, medial preoptic nucleus; VMN, ventromedial hypothalamic nucleus.

influenced by selection on the interaction between aggression and reproductive success, and in species where aggression does not influence reproductive success male VMN volume will not be influenced by sexual selection.

The VMN facilitates female reproductive behaviour (Goodson, 2005; Numan, 2014), and if our indices of sexual selection reflected the strength of sexual selection in both sexes, then we would expect female VMN volume to be positively related to one or more indices. However, female VMN volume was not predicted by any index of sexual selection. This suggests that perhaps our indices of precopulatory sexual selection do not reflect the strength of sexual selection in females. This is consistent with mate-choice experiments in lizards generally (Tokarz, 1995) and in three species of *Ctenophorus* in particular (LeBas & Marshall, 2001; Olsson, 2001; Jansson *et al.*, 2005) which found no evidence for female mate choice. Female choice in *Ctenophorus* might be post-copulatory, and therefore physiological rather than behavioural, thereby precluding sexual selection on the brain.

We selected the DMT and DLH as controls because they are also diencephalic and are therefore anatomically and developmentally proximate to our regions of interest. However, neither is known to be involved in sexual behaviour, reward (which is closely associated with sexual behaviour), or social behaviour. The DMT is a thalamic relay which receives projections primarily from the spinal cord and raphe and projects

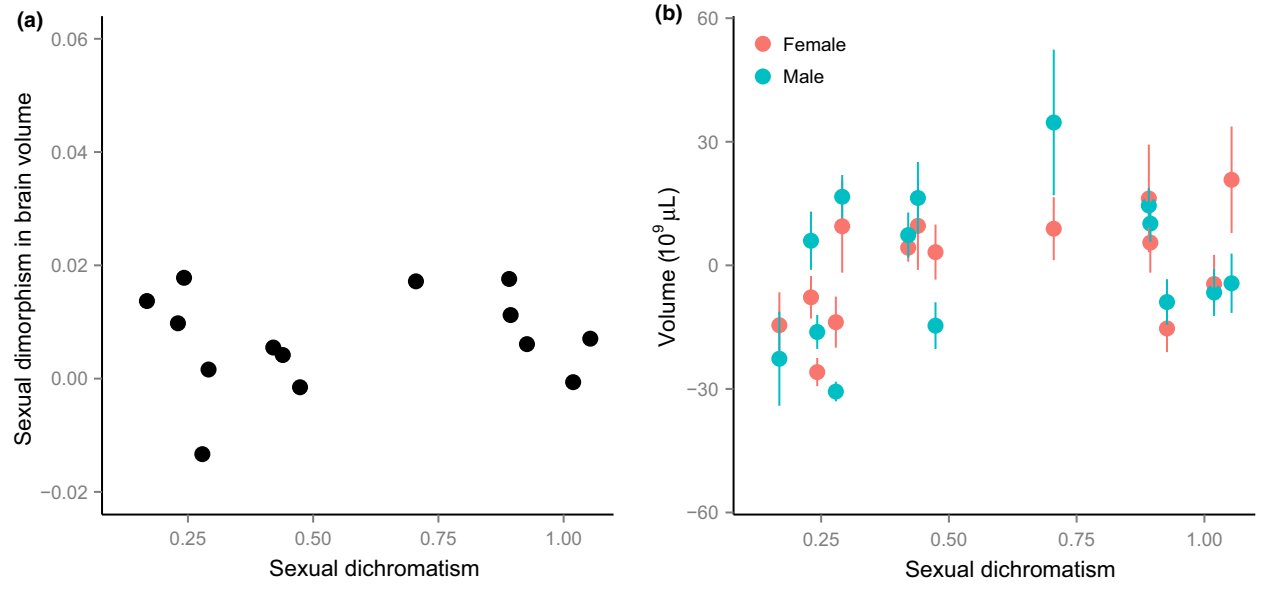

Fig. 4 Variation in brain volume (BV) was not related to sexual dichromatism, an index of sexual selection. Neither sexual dimorphism in BV (a) nor the BV of males and females (b) shows any variation in response to the strength of sexual selection. In (a), points are sexual dimorphism values (*d*) for each species. In (b), points show size-independent species means ± standard error.

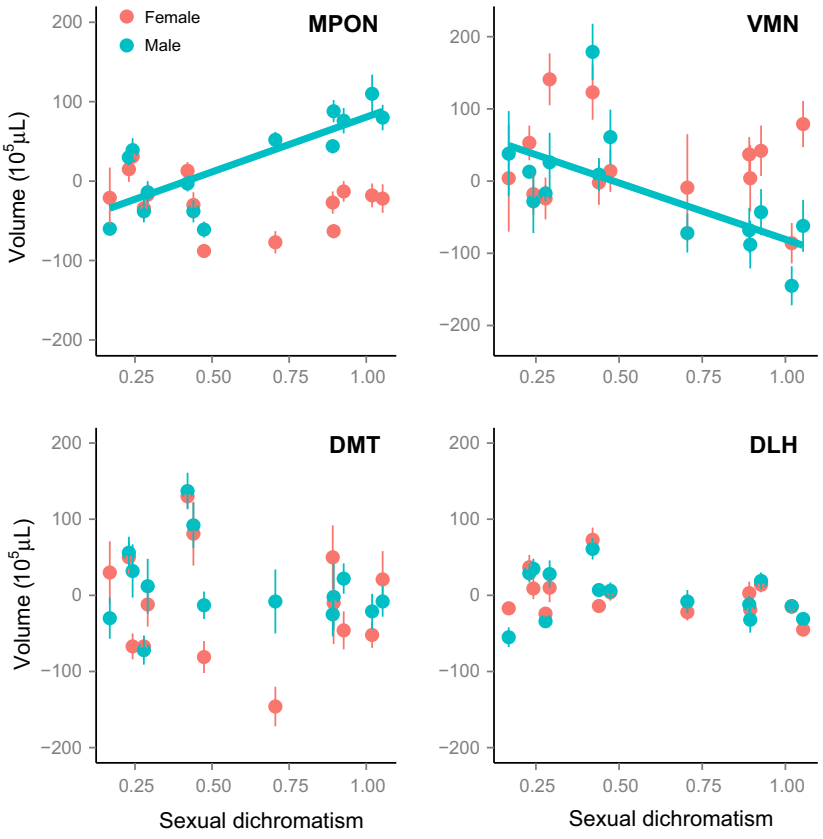

Fig. 5 The volumes of the MPON, VMN, DMT and DLH in males and females have a variety of different relationships with sexual dichromatism (SDC), an index of sexual selection. Male MPON volume is positively correlated with SDC, whereas male VMN volume is negatively correlated. Male DMT and DMH volumes and all female volumes do not correlate with SDC. Points show size-independent species means ± standard error. Regression lines are shown for relationships that have a nonzero slope. DLH, dorsolateral hypothalamic nucleus; DMT, dorsomedial thalamic nucleus; MPON, medial preoptic nucleus; VMN, ventromedial hypothalamic nucleus.

Table 4 Phylogenetically corrected standard major axis regressions. Each brain structure that was shown to be correlated with BV in the averaged linear model estimates is tested for concerted evolution with respect to BV. $P > 0.05$ in all cases, indicating concerted evolution.

Sex	Brain Region	Intercept	Slope	r^2	T	d.f.	P
Male	MPON	1.656	1.075	0.225	0.283	12.779	0.781
	VMN	2.749	0.955	0.543	0.238	11.436	0.816
	DMT	2.272	0.944	0.635	0.860	10.453	0.409
	DLH	2.943	0.905	0.839	0.332	11.107	0.746
Female	MPON	–	–	–	–	–	–
	VMN	2.459	1.015	0.791	0.114	10.599	0.912
	DMT	2.399	1.007	0.651	0.042	11.994	0.967
	DLH	1.523	1.091	0.401	0.392	11.053	0.702

BV, brain volume; DLH, dorsolateral hypothalamic nucleus; DMT, dorsomedial thalamic nucleus; MPON, medial preoptic nucleus; VMN, ventromedial hypothalamic nucleus.

primarily to the telencephalon, including the cortex, dorsal ventricular ridge and striatum (ten Donkelaar & De Boer-Van Huizen, 1988; Butler, 1994). The DLH is a component of the thalamic reticular nucleus, a region of unknown function in reptiles but involved in the control of sleep in mammals (Díaz *et al.*, 1994; Dávila *et al.*, 2000; Desfilis *et al.*, 2002). Although we did not find that either of these regions changed in volume with the strength of sexual selection, we do note that there is variation in sexual dimorphism in these regions between species (Fig. 3), and we suggest two potential causes. First, this could be due to complex interactions between natural and sexual selection that influence brain evolution, such as our hypothesis that VMN variation in males may be due to the interaction between selection on aggressive behaviour and sexual selection. Another explanation is ecological divergence, where differences between sexes evolve due to divergent natural selection, that is sexes adapt to different ecological niches (Shine, 1989). It should be noted that ecological divergence should be considered as a possible alternate explanation for MPON and VMN evolution as well. However, we consider ecological divergence an unlikely explanation as this process has not been documented in *Ctenophorus*, although it is poorly understood in lizards (Shine, 1989). Too little is known about the social behaviour and ecology of *Ctenophorus* to tease apart these complex interactions, although *Ctenophorus* is among the most intensely studied genera of lizards. Studying the complex ways natural and sexual selection interact to shape the brain is an exciting but very challenging area of future research (Cahill & Aswad, 2015).

We have also found evidence that the MPON, VMN, DMT and DLH are evolving in concert as they show the same pattern of variation with respect to the volume of the entire brain. BV is an emergent property of the evolution of various brain components and may not have a direct function. Nonetheless, selection may act directly on BV as a whole. For example, the brain is energetically demanding and therefore places a disproportionate burden on the organism compared to other tissues (Pitnick *et al.*, 2006). Evolution may exert selective pressures on BV to minimize this burden. The nuclei we measured are small compared to the volume of the entire brain (Fig. 2), and variation in their size is unlikely to significantly influence BV. However, the reverse is likely true: BV is an emergent property of the nuclei that make it up and as such selection on BV is likely to influence the variation in the individual nuclei that make up the brain.

Ultimately, we have found that the MPON, VMN, DMT and DLH are evolving both in concert and as a mosaic. This is consistent with the prevailing view in mammals and birds: the brain is undergoing both mosaic and concerted evolution simultaneously in response to different selective pressures (Striedter, 2005; Gutiérrez-Ibáñez *et al.*, 2014; Herculano-Houzel *et al.*, 2014; Corfield *et al.*, 2015). Although concerted brain evolution has previously been described in *Anolis* (Powell & Leal, 2012, 2014), to our knowledge, this study is the first to find evidence for mosaic brain evolution in a reptile. Sexual selection has likely played an underappreciated role in organizing the brain (Cahill & Aswad, 2015) and future studies addressing this deficit could be especially rewarding and enrich a rapidly growing field.

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Authors' contributions

This project was conceived by DH, MJW and JSK. DH, JFPU, MJW and JSK designed the research. DH and JAE designed the colour aspect; DH collected the data which was analysed by JAE. DH, TSG, YD and WSP conducted the MRI scanning, and DH, JFPU and ALJ analysed the MRI images. DH conducted the histology. DH, MVG, TM and JSK conducted the data analysis. DH, MJW and JSK drafted the manuscript with JAE, TSG and ALJ contributing method components. All authors contributed to editing the manuscript and gave final approval for publication. Authors declare no conflicts of interest.

Data archiving

Raw data are included as an electronic supplementary material.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Table S1 Means and standard errors for each brain region per sex/species.
Table S2 Averages of Phylogenetic Linear Models for each brain region, using various AICc cut-off values.
Table S3 Raw data used for the analyses conducted in this paper.

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