

Mating system variation and morph fluctuations in a polymorphic lizard

MATS OLSSON,* MO HEALEY,* ERIK WAPSTRA,† TONIA SCHWARTZ,* NATASHA LEBAS‡ and TOBIAS ULLER*

*School of Biological Sciences, University of Wollongong, NSW 2522, Australia, †School of Zoology, The University of Tasmania, Hobart, Tas. 7001, Australia, ‡Center for Evolutionary Biology, School of Animal Biology (M092), The University of Western Australia, Crawley, WA 6009, Australia

Abstract

In polymorphic male painted dragon lizards (*Ctenophorus pictus*), red males win staged contests for females over yellow males, and yellow males have greater success in staged sperm competition trials than red males. This predicts different reproductive strategies in the wild with red males being more coercive or better mate guarders than yellow males. Yellow males would be expected to sire more offspring per copulation and have a greater proportion of offspring from clutches with mixed paternity. However, here we show using microsatellites that the frequency of mixed paternity in the wild is low (< 20% on average across years), that all morphs on average have the same number of offspring sired per year, and that mating system variation (polyandry vs. monandry) is strongly correlated with perch density on male territories. Furthermore, a logistic regression on male successful vs. unsuccessful mate acquisition showed that red males were under negative selection when they dominated the population, which suggests ongoing frequency dependent selection on male colouration.

Keywords: lizard, male polymorphism, mating system, reproductive success in the wild, territoriality, vigilance

Received 25 July 2007; revision received 5 August 2007; accepted 11 September 2007

Introduction

The evolution of polymorphism is a classic problem in evolutionary ecology, representing a wide range of taxa (e.g. Darwin 1859; Gross 1996; Schuster & Wade 2003), all with the inherent problem of how and why multiple morphs co-exist over evolutionary time in the wild. The most common explanation is that morphs are condition-dependent and change through life at a switch point when alternative morphs have equal fitness (Gross 1996; but see Schuster & Wade 2003). Alternatively, if morphs are entirely genetically determined, they may co-exist at equal frequency when the individuals have equal fitness, or different frequencies that offset their fitness differences (Schuster & Wade 2003). Another explanation is that morphs may cycle in frequency over time, where differences in reproductive tactics may

select for a particular morph under certain conditions. The most elegant example of this is the Uta lizard (*Uta stansburiana*), in which three morphs cycle in frequency depending on how they may invade the dominant current strategy (the rock-paper-scissors game; Sinervo & Lively 1996). In this species, blue males are mate guarders and have a higher probability of paternity than orange males that have large territories with many females, whereas yellow males share paternity primarily with orange males (Zamudio & Sinervo 2000). This occurs in a system with very high levels of mixed paternity (60–80%; Zamudio & Sinervo 2000), in which the morph cycles are driven by the numerical dominance of one morph, while leaving themselves open to invasion by a morph 'specialized' for the current situation. For instance, when orange males dominate the population, yellow males are expected to invade with a sneaker strategy.

We studied the Australian painted dragon lizard (*Ctenophorus pictus*), which also occurs in three different morphs (red, yellow and orange, which we have referred to as two different morphs elsewhere, red and yellow, since

Correspondence: Mats Olsson, Fax: +61 24221 4135; E-mail: molsson@uow.edu.au

orange males occurred in extremely low frequencies at the identification of our model system; Healey *et al.* 2007). Adult males captured post-emergence from hibernation show the same head colour throughout the breeding season. Thus, since males are annual (less than 10% of males survive a second hibernation), we can be confident that their morphs do not change through life. Females have the same head as body colour (reddish-brown, camouflaged) and attempts to express the sex-limited colour that females are genetic carriers for using testosterone has unfortunately failed. Our evidence, however, suggest that male head colour is a genetically determined, sex-limited trait that does not change throughout a male's life time and is not subject to conditional effects within morphs (e.g. changing from one colour to another if males become better or worse in body condition). Elsewhere, we report on a high-performance liquid chromatography (HPLC) and acidified pyridine analysis (M. Olsson, T. Schwartz, T. Uller & M. Healey, submitted), in which we analyse the underlying physiological mechanism to the red and yellow colours (orange not analysed). In summary, this analysis showed that yellow colour is determined by integumental deposition of carotenoids, whereas red colour is also determined by deposition of another (yet to be identified) pigment, most likely a pteridine (M. Olsson, T. Schwartz, T. Uller & M. Healey, submitted). The morphs do not differ in body size (e.g. snout-vent length, mass or head length and width, Healey *et al.* 2007; further results in this manuscript), territory size, or the number of perch sites found on male territories (see Results for additional information).

After hibernation in burrows under ground, males and females emerge in late August to early September, when males establish territories (Cogger 2000; Healey *et al.* 2007). All male morphs are observed at the same rate in the wild (see Results), suggesting that neither morph adopts a strategy that relies on particularly inconspicuous behaviour. Because of the rarity of the orange morph in the wild at the onset of our study (< 15% of the population at the time), previous assessment of behavioural differences among the morphs has only involved the red and the yellow morphs. Red males win staged contests over females more frequently than yellow males (Healey *et al.* 2007). Yellow males, however, sire three times more offspring in staged sperm competition trials in which females have first mated with the male on whose territory they reside in the wild (M. Olsson, T. Schwartz, T. Uller & M. Healey, submitted). Thus, this suggests that males have different reproductive strategies in the wild and are favoured by selection under different conditions. Furthermore, since we have observed dramatic changes in the morph frequencies since 1999, we include descriptive data of morph year-to-year variation.

In conclusion, our morphometric data on wild and captive males, and our laboratory data on male behaviour, suggest that males of different morphs may employ differ-

ent reproductive tactics but none of these are related to apparent phenotypic differences among morphs or to conspicuous behaviours. The latter results caution against formulation of explicit predictions at this stage of investigation. Instead, we formulated four analytical goals with this study. (i) Describe inheritance patterns of male colouration using a limited data set from a breeding experiment (to study sperm competition, results submitted elsewhere), for which hatchling males were kept until they expressed adult colouration and whose fathers were assigned using microsatellites (following the same protocol as in the present study, see Materials and Methods below). The sons in this data set all came from the first clutches produced during the season, since these were the only ones held in captivity for long enough to develop adult breeding colouration before autumn release. (ii) Describe changes in the frequencies of the morphs through 5 years of data during which males were sampled at random in the wild. (iii) Paternity-assign offspring in the 2 years for which the lizards were monitored every day and tissue for molecular paternity assignment was collected. This data set was then used to investigate morph-specific correlates of reproductive success in the wild in order to test whether males of different morphs sired more (or less) offspring under morph-specific conditions. In particular, we aimed to test whether yellow males sired relatively fewer young with multiple females than red males did (suggesting a sneaker strategy), rather than have a higher probability of paternity in a single clutch (typically expected to be a female on a red male's territory). (iv) Calculate morph-dependent selection gradients in order to assess frequency-dependent selection in the wild.

Materials and methods

Painted dragon lizards (*Ctenophorus pictus*) are small (up to 16 g) lizards that live in desert country in Southeast Australia. We studied a population in Yathong Nature Reserve, New South Wales (145°35'E, 32°35'S) by full-time monitoring (2000 and 2005) or by random sampling of lizards to estimate morph frequencies (1999, 2004, 2006). Lizards were noosed upon their emergence from hibernation (hereafter 'day of first capture') and observed from a slow-moving (and stopping) vehicle along a fire trail and reserve boundary. The habitat in this area is dominated by sand dunes with tree stumps and shrubs used by the lizards as perch sites during territorial vigilance. In the 2000 and 2005 field seasons, the population was monitored every day (weather permitting lizard activities) from late August to late December, that is the majority of the mating season (which may continue into January in relatively cooler years; Harlow, personal communication). More detailed field protocols have been published elsewhere (Healey *et al.* 2007), so we only give a brief account of essential techniques here. Captured lizards were weighed, measured

(only relevant measures reported), and blood sampled (from *v. angularis* in the corner of the mouth), scored for head colouration by eye (following Sinervo & Lively 1996), and had a number painted on their backs before being released at the place of capture. Blood or toe/nail clips were stored in 96% ethanol until DNA extraction. Lizard observations were global positioning system (GPS)-recorded along the territories, which are close to one-dimensional on the embankments of the fire trails forming our study site; vegetation is too dense on the sides of the embankments and the open road typically discourages lizard activities (probably because of risk of predation). Thus, although males occasionally take refuge from predators in the surrounding vegetation, they typically patrol their territories only from perch sites along the trail embankments and we therefore used the longest axis of their territories as a proxy for territory size. The number of perch sites higher than knee-height were counted between the GPS end points of each territory at the end of the mating season. However, for logistic reasons, in 2005 only a subsample ($n = 37$) of the total number of male territories ($n = 100$) were surveyed.

Once females showed egg contours, they were brought into oviposition cages with a spotlight for thermoregulation to their preferred body temperature (c. 36 °C) arranged at one end of their cage and with a watered patch to direct oviposition without egg desiccation. Once oviposition began in captivity, eggs were harvested twice a day and incubated at ideal incubation temperatures (30 °C) until hatching (c. 60 days). Hatchlings were weighed and measured and had a c. 3-mm tip of the tail removed for genotyping. They were then released at random sites at Yathong Nature Reserve.

We analysed a set of traits previously reported as significant in studies of reproductive success, or successful mate acquisition in territorial lizards (Olsson & Madsen 1998), or that seemed relevant from a perspective of painted dragon reproductive biology (Healey *et al.* 2007). These variables were mass, snout-vent length (SVL), body condition (residuals from a mass-SVL regression), day of first capture, territory size, number of perch sites on the territory, a colour competition index of neighbours (proportion red males, since these are the most aggressive), and a male's reproductive success in absolute number of paternity-assigned young.

In order to be able to calculate selection coefficients (following Janzen & Stern 1998) on a data set including males that did not sire offspring, but without penalizing males whose females may never have been caught, we assigned reproductive success for males that were physically present around a female whose offspring we successfully assigned with microsatellites. The limit for including the males surrounding her was set to the maximal distance a male was separated from a female with which he sired some or all of her offspring (100 m).

Parentage analysis was performed on DNA samples from years 2000 and 2005 using microsatellite markers, and parentage assignment in CERVUS 2.0 (Marshall *et al.* 1998). DNA was isolated from blood samples (year 2000) and toe or tail clips from year 2005 using a QIAGEN DNeasy Tissue Extraction Kits (QIAGEN). DNA samples from 2000/2005 were polymerase chain reaction (PCR) amplified at the six microsatellite loci CP01, CP02, CP06, CP10, CP11, and CP17 (Schwartz *et al.* in press), using fluorescently labelled primers. The samples from 2005 were amplified at the following eight microsatellite loci, CP01, CP02, CP06, CP10, CP11, CP17, AM41 (Schwartz *et al.* in press), and C7 (Austin *et al.* 2006) using fluorescently labelled primers. All loci were amplified in 7- μ L reactions either singleplexed or multiplexed using 10–20 ng of DNA; 0.22 mM each dNTP; 0.3–0.7 μ M of each primer; 1 \times PCR buffer (QIAGEN) containing Tris-HCl, KCl(NH₄)₂SO₄, 15 mM MgCl₂ pH 8.7; additional MgCl₂ (final concentration of MgCl₂ ranged from 1.5 to 3.5 mM, depending on the loci being amplified); and 0.05 U of HotStart Taq (QIAGEN). Cycling conditions included a hotstart denaturation at 95 °C for 15 min; 30 cycles of 95 °C for 30 s, annealing temperature for 30 s, 72 °C for 30 s; and a final extension at 72 °C for 30 min. Fluorescent amplifications were electrophoresed on a genetic analyser (ABI 3130xl, year 2000 and ABI 3900, year 2005, Applied Biosystems) using the LIZ-500 size standard (Applied Biosystems). Alleles were scored using GENEMAPPER (Applied Biosystems) and confirmed visually.

Parentage analyses were conducted independently for each year using both exclusion and likelihood-based approaches in CERVUS 2.0 (Marshall *et al.* 1998). Genotypes from the adults were used to calculate allele frequencies, observed and expected heterozygosities, frequency of null alleles, and the polymorphic information content (PIC, a measure of informativeness related to expected heterozygosity) of the loci (Botstein *et al.* 1980; Hearne *et al.* 1992; Schwartz *et al.* in press; Table 1). Mother-offspring genotypes were compared for the presence of null alleles and loci containing null alleles were deleted from the analyses. All males sampled in the population were assumed to be potential fathers for all offspring in the year they had been sampled (except for five individuals in 2005 that had less than four loci genotyped). For these analyses, we assumed that 80% of the males in the population were sampled. To determine 95% and 80% confidence levels for the parentage assignments, two types of simulations were run on each data set: the first assumed a zero genotyping error rate (complete exclusion), and the second assumed a 0.01 genotyping error rate. The data sets were then corrected for any potential null alleles by deleting all homozygous genotypes at loci CP02, CP10, CP17, and AM41 (loci most likely to have null alleles) from the potential fathers and assigning parentage a third time using a zero genotyping error rate simulation. Results from all three analyses were

Locus	Year	No. of alleles	PIC	Excl	H_O	H_E	Null freq
CP01	99/00	17	0.839	0.715	0.879	0.858	-0.013
	05/06	16	0.824	0.695	0.818	0.841	0.015
CP02	99/00	25	0.925	0.858	0.941	0.934	-0.006
	05/06	29	0.926	0.860	0.851	0.933	0.045
CP06	99/00	39	0.960	0.920	0.954	0.966	0.003
	05/06	52	0.959	0.920	0.914	0.963	0.025
CP10	99/00	39	0.958	0.918	0.863	0.965	0.054
	05/06	51	0.961	0.925	0.872	0.965*	0.050
CP11	99/00	16	0.813	0.675	0.832	0.837	0.003
	05/06	15	0.771	0.620	0.793	0.794	-0.003
CP17	99/00	35	0.946	0.895	0.856	0.953	0.953
	05/06	43	0.923	0.857	0.770	0.929*	0.094
AM41	05/06	36	0.917	0.844	0.848	0.924	0.042
C7	05/06	8	0.754	0.582	0.766	0.788	0.009
Mean	99/00	28.5	0.907	0.830			
	05/06	31.25	0.879	0.788	0.829	0.892	
Total	99/00			0.999			
Exclusionary Power	05/06			0.999			

Table 1 Power of loci to identify parentage as calculated in CERVUS 2.0. PIC represents the polymorphic information content; excl represents the power of the locus to exclude a randomly selected male from being the father of a randomly selected offspring based only on the offspring and the mother's genotypes; H_O and H_E represent the observed and expected heterozygosities with * designating loci violating HWE; null freq represents the estimated frequency of null alleles at that locus

cross-checked and assigned fathers were verified by eye across mother-offspring pairs and the clutchmates. Any mismatches between father-offspring-mother were checked with the raw data.

Based on the loci used, the probability of exclusion when the mother's genotype was known was 0.999 for both years' data sets. Simulations estimated that with the known mother's genotype, paternity could be successfully assigned at 95% confidence in 80% of cases with complete exclusion in both years (only 80% because we conservatively assumed we had only sampled 80% of males in population). Assuming a 0.01 error rate, paternity was estimated to be successfully assigned at the 95% confidence level in 62% and 64% of cases in 2000 and 2005, respectively. The fathers that were identified after comparing the three types of analyses never had more than one allele mismatch and always fell within the 95% confidence level or the 80% confidence level with siblings having been assigned the same father at the 95% confidence level.

Results

Laboratory study of inheritance of colouration

Forty-one sons with expressed adult colouration from a total of 19 clutches and 21 sires were used to investigate patterns of inheritance of colouration (Table 2). Below, we evaluate three patterns of inheritance using defined rejection criteria (under the assumption that colouration is a sex-limited trait, and dam genotype is unknown).

One-locus, two-allele, codominance model. According to this model, red males have genotype RR, orange males RY, and yellow males YY. If true, red males can only sire red or orange sons, yellow males can only sire yellow and orange sons, and orange males can sire sons of all three colour morphs. Indeed, orange males sire all three morphs, even within the same clutch (sire 45, dam 45), and all red males sired red sons exclusively (e.g. sire 34, 54, 152). However, six different yellow males sire seven red sons in six different clutches (sire 25, 151, 201, 221, 230, 320). Thus, the latter observation rejects this pattern of inheritance.

Polygenic inheritance. In order to test polygenic inheritance of colouration, we assigned 1, 2, 3 as numerical scores for yellow, orange, and red, respectively, and regressed mean son scores on the paternal score. This regression analysis (and hence heritability for colour) was nonsignificant ($F_{1,18} = 2.18$, $P = 0.16$, $R^2 = 0.11$). Even though red males produced nothing but red sons (see above), yellow males' production of red sons strongly contributed to this nonsignificant outcome.

Single-locus, three-allele model. In this model, R is dominant to O, which is itself dominant to Y. Thus, red males are RR, RY, or RO, orange males are OO or OY, and yellow males are YY. Any sire can produce any colour son, depending on the genotype of his mate. A test of our empirical son data against the frequencies theoretically predicted under this model is clearly confounded by clutch size. Therefore, we refrain from a formal test and only report the raw data,

Table 2 Inheritance of colour in painted dragon males

Son colour	Sire ID	Sire colour	Dam ID
red	13	orange	330
yellow	25	yellow	44
yellow	25	yellow	44
red	25	yellow	235
red	34	red	215
yellow	45	orange	45
red	45	orange	45
orange	45	orange	45
yellow	45	orange	45
red	45	orange	45
red	45	orange	45
red	45	orange	220
red	53	orange	330
red	54	red	22
yellow	104	orange	103
yellow	104	orange	132
yellow	110	yellow	105
red	131	orange	12
red	151	yellow	113
red	152	red	123
red	152	red	123
red	152	red	123
red	201	yellow	232
red	221	yellow	221.3
red	230	yellow	32
red	230	yellow	32
red	315	orange	232
red	320	yellow	22
yellow	320	yellow	235
red	323	orange	31
red	333	orange	12
red	333	orange	12
red	333	orange	12
red	333	orange	12
red	333	orange	12
red	333	orange	12
red	333	orange	12
red	333	orange	12
red	333	orange	220
red	335	red	105

which was 32:1:8 (red: orange: yellow sons), produced from six, six, and seven sires, respectively, of the corresponding morphs. We also performed chi-squared tests of our counts of each morph against those predicted by Hardy–Weinberg equilibrium allele frequencies for the pooled data set across years, and for the year (2005) for which we had most data. Both these frequencies deviated significantly from those predicted under Hardy–Weinberg equilibrium under this inheritance model. For the full data set, the predicted counts of red, orange and yellow males were 66, 95 and 104. A test against the summed counts in Fig. 1 yield a χ^2 of 39.8 ($P < 0.001$). The corresponding

expected counts for year 2005 was 45, 30 and 25, which yield a $\chi^2 = 8.0$ ($P < 0.02$) when tested against those empirically found.

Determinants of reproductive success in the wild

To set the scene, we present the frequencies of the three morphs from the 5 years of sampling, including when we did not continuously monitor the lizards, and conclude that morph frequencies vary drastically across years (Fig. 1; e.g. red males vary in frequency from 14% to 57%; Fig. 1). We also add rainfall data to Fig. 1 as a proxy for how benign conditions were, varying from rich conditions in 1999 (obvious to an observer from the abundance of flowering plants), to drought conditions in 2004–2006 (an arid zone, Australia-wide crisis during these years). A Pearson's chi-squared test confirmed that the morph frequencies changed significantly among years (Monte Carlo simulated analyses, 10 000 samples, $\chi^2 = 41.9$, $P < 0.001$; STATXACT 7, Cytel).

Male mass, snout-vent length, number of observations, territory size, and number of perch sites on a male's territory did not vary significantly among male colour morphs (Proc GLM, SAS; unbalanced two-factor analyses of variance, with year and head colour as factors; $n > 60$, $P > 0.30$ for all comparisons; Appendix, Supplementary material).

An analysis of variance on pooled data for both years revealed no difference in reproductive success among colour morphs (model $F_{2,89} = 0.11$, $P = 0.89$, $R^2 = 0.002$, Type III SS = 1.13, MS = 0.57, head colour as fixed factor). However, since our aim is to analyse ongoing selection in the wild during biologically relevant episodes, and previous analyses of polymorphic systems have revealed strong year-to-year differences in reproductive success among morphs in a cyclic manner (Sinervo & Lively 1996), we proceeded with year-specific analysis. Descriptive data and sample sizes of traits with significant differences between morphs in at least 1 year are presented in Table 3, in addition to data on reproductive success from the 2 years for which we have data.

Male colour competition index varied significantly among male colour morphs in 2000 ($F_{2,44} = 4.21$, $P = 0.022$), but not in 2005 ($P > 0.22$), with orange males having twice as high an index as red and yellow males (suggesting that orange males are more surrounded by red males than are the other morphs; Table 3). Male day of first capture did not differ significantly among morphs in 2000 ($P > 0.11$), but was highly significantly different among morphs in 2005 ($F_{2,98} = 10.99$, $P < 0.0001$; Table 3), with red males emerging on average 23 days before orange males, and 17 days before yellow males. Correspondingly, body condition did not differ among morphs in the more benign year, 2000 ($P > 0.45$), whereas this difference was statistically significant in 2005 ($F_{2,98} = 6.77$, $P = 0.002$), with red males being much thinner on average than both other morphs (Table 3).

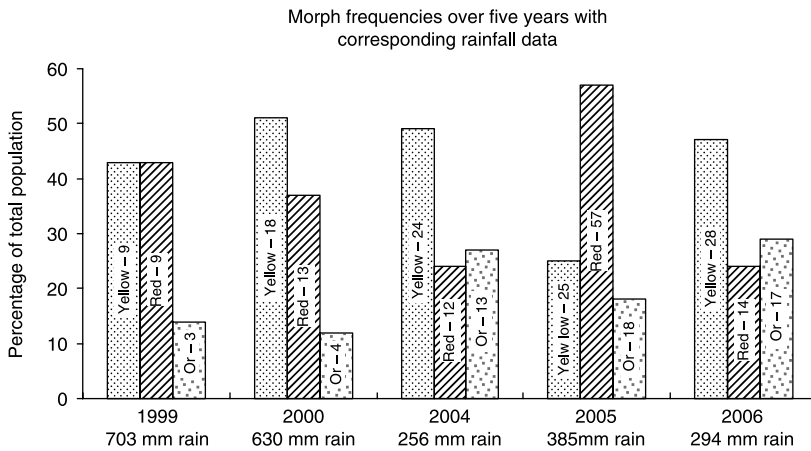


Fig. 1 The figure describes the change in morph frequencies during years for which we monitored a population of painted dragon lizards (*Ctenophorus pictus*) throughout the mating season (1999/2000, 2005/2006), or sampled the same population to assess changes in morph frequencies. Rainfall is given in millimetres to provide information on change in environmental conditions. For illustration purposes, only the starting year of a field season is given in the figure, even though the season may continue into the following year during the southern hemisphere summer.

Table 3 Descriptive statistics for the three different head colour morphs from 2 years of data for which we assigned reproductive success with microsatellites. Means \pm SE (standard errors) are given in addition to sample sizes

	2000 Mean (\pm SE)			2005 Mean (\pm SE)		
	red	orange	yellow	red	orange	yellow
Colour competition index (percentage of red neighbours)	0.32 \pm 0.03 <i>n</i> = 26	0.62 \pm 0.02 <i>n</i> = 3	0.38 \pm 0.05 <i>n</i> = 16	0.35 \pm 0.03 <i>n</i> = 54	0.43 \pm 0.04 <i>n</i> = 19	0.42 \pm 0.05 <i>n</i> = 24
day of first capture	253.4 \pm 1.07 <i>n</i> = 26	260.0 \pm 5.13 <i>n</i> = 3	252.7 \pm 1.18 <i>n</i> = 18	268.1 \pm 2.97 <i>n</i> = 55	291.3 \pm 3.96 <i>n</i> = 19	285.5 \pm 4.56 <i>n</i> = 25
Body condition (residuals mass-svl regression)	0.10 \pm 0.16 <i>n</i> = 26	-0.59 \pm 0.36 <i>n</i> = 3	-0.04 \pm 0.24 <i>n</i> = 18	-0.42 \pm 0.15 <i>n</i> = 55	0.44 \pm 0.29 <i>n</i> = 19	0.59 \pm 0.32 <i>n</i> = 25
reproductive success	1.78 \pm 0.67 <i>n</i> = 18	2.0 \pm 1.15 <i>n</i> = 3	1.69 \pm 0.75 <i>n</i> = 13	1.5 \pm 0.36 <i>n</i> = 36	1.89 \pm 0.45 <i>n</i> = 9	1.46 \pm 0.48 <i>n</i> = 13

Thus, the main differences among morphs appeared to be in day of first capture and body condition, and these results are significant also after Bonferroni correction for multiple testing (i.e. adjusting for two tests, on condition and day of first capture, on the same data set; $\alpha = 0.01$). Finally, average reproductive success varied between 1.46 and 2.00 young among years and morphs (Table 3) but was not significantly different among morphs in either of the two years ($F_{2,31} = 0.02$, $P = 0.98$, vs. $F_{2,57} = 0.16$, $P = 0.86$ for 2000 and 2005, respectively).

We then examined male reproductive success in relation to how paternity was acquired through singly- or multiply-sired clutches. In 2000, we successfully determined the paternity of 18 (of 26) clutches comprising 62 young. Of these 18 clutches, one was multiply sired (5%). In 2005, we successfully determined the paternity for 33 (of 39) clutches comprising 110 young. Of these 33 clutches, eight were multiply sired (24%).

Under the assumption that the density of perch sites on a male's territory facilitates the exclusion of trespassing rivals through increased vigilance, we would predict that males who gain their reproductive success through singly-sired clutches hold territories with higher perch densities

than those territories resulting in multiply-sired clutches. This turned out to be the case. Monandrous clutches came from territories with on average three times higher perch density than polyandrous clutches [mean number of perch sites, 4.75/m \pm 0.56, SE, vs. 1.63/m \pm 0.23, SE, respectively; two-sample *t*-test, d.f. = 52.9 (Satterthwaites' approximation), $t = 7.2$, $P < 0.0001$]. To analyse this from a different perspective, we entered perch density (covariate) and head colour (factor) from the pooled data set into a logistic regression and analysed whether perch density per se would predict mating system (monandry vs. polyandry). This analysis also confirmed the significant effect of perch density on mating system (Wald $\chi^2 = 8.1$, d.f. = 3, $P = 0.0043$, with no significant effect of head colour, $P > 0.10$). Furthermore, red males resided on territories with a higher average perch density than yellow males [both years pooled for analysis, mean perch density, 0.98/m \pm 0.09 vs. 0.70/m \pm 0.09; two-sample *t*-test, $t = 2.10$, d.f. = 45.6 (Satterthwaites' approximation), $P = 0.042$], whereas the corresponding difference between orange and yellow males was nonsignificant, even though the average perch density for the few orange males we had data for ($n = 5$) was in fact even larger than for red males [1.24/m \pm 0.33, vs. 0.70/

$m \pm 0.09$, two-sample t -test, $d.f. = 4.59$ (Satterthwaites' approximation), $t = 1.56$, $P = 0.18$]. The proportion of males that acquired offspring through multiply-, compared to singly-sired clutches did, however, not differ among morph categories ($\chi^2 < 1.27$, $P > 0.53$, $d.f. = 2$ in both years).

Most males that sired offspring did so with one female only, whereas one male in 2000 sired offspring with two females and another male in the same year with three females. In 2005, five males sired offspring with two females and one male with three females. In the majority of clutches (85%), males sired offspring with a single female on his territory. We therefore proceeded with a logistic regression analysis (binary distribution, logit link function) in which we assessed maximum-likelihood estimates of selection gradients with respect to male successful vs. unsuccessful mate acquisition, starting with a model including male head colour as a fixed factor, and day of first capture and male condition as covariates. Male condition was dropped from the final models (backwards elimination at $P > 0.25$) and we therefore only report the models including morph colour and day of first capture. Following Quinn & Keough (2003; p. 370), the significance level of head colour was assessed by running the logistic regression model twice, with and without the fixed factor, and performing a likelihood ratio test by subtracting the two -2 Log L scores and comparing the resulting difference against the critical χ^2 value for one degree of freedom (which equals 3.84). This test was nonsignificant in 2000 (LR = 2.72, $P > 0.05$), but was significant in 2005 (LR = 5.4, $0.025 > P > 0.01$), with a positive parameter estimate for orange males (1.11 ± 0.62) and a negative one for red males (-0.91 ± 0.45). Mean first day of capture did not reach significance in either year ($P = 0.08$, and $P = 0.24$, in the 2 years, respectively).

Discussion

Our data show that males have the same colour through life. However, the inheritance pattern of male colouration in painted dragons is complex, qualitatively rejecting a simple two-allele, codominance model and casting doubt on a polygenic inheritance model. However, one model cannot be dismissed with the data at hand, a single-locus three-allele model. The most consistent result was that red sires only produced red sons, and the overproduction of red sons compared to the other two morphs, primarily from orange sires (although clutch size is a confounder in our analysis). We do not know the underlying explanation to this, but make the observation that red males emerge much earlier from hibernation than the other morphs in some years. If the corresponding phenomenon occurs in females of the same genotype, that is red homozygous females emerge earlier from hibernation than any other female genotype, then we are likely to have over-represented these females in our breeding experiment. Male territorial

agamid lizards tend to emerge earlier than females from hibernation in order to set up territories (Cogger 2000) and we started sampling lizards for our laboratory trials as soon as lizards emerged. Thus, even if we sampled males for long enough early in the season to obtain specimens of all morphs, our sampling of females may have been (strongly) biased towards genetically red females and with many more sons produced from crossings with genetically red females than any other female genotype. This scenario is entirely congruent with the over-representation of red sons in our breeding trials. Potentially, this may have important implications for how morph frequencies change between years, in particular when the changes in frequencies are much more rapid than can be logically inferred from a selection argument. A test of this hypothesis would require sampling of males and females throughout the season and a more in-depth genetic analysis of the inheritance patterns of colour morphs through the temporal axis of the mating season. Such phenomena may also explain the deviation of morph frequencies from those predicted by the Hardy–Weinberg law under the assumptions of the inheritance model we found support for. Such deviations are also expected if there is ongoing frequency dependent selection, which our results suggest.

We have shown significant differences in behaviour between the red and yellow morphs in captivity (orange males were never tested in this regard), showing red dominance over yellow males more frequently than expected by chance (Healey *et al.* 2007), and a yellow male advantage in sperm competition trials (M. Olsson, T. Schwartz, T. Uller & M. Healey, submitted). However, we could not verify strong and straightforward differences in morph-specific reproductive tactics in the wild that would have been predicted from our trials in captivity, unlike in the work on *Uta* lizards (*Uta stansburiana*; Sinervo & Lively 1996; Zamudio & Sinervo 2000). A contributing factor to this difference in results could be habitat constraints on the evolution and expression of morph-specific behaviour and their evolutionary divergence in painted dragons. The long-term studies of *Uta* lizards by Sinervo and colleagues takes place on rocky outcrops with a complex three-dimensional habitat (Calsbeek & Sinervo 2002a), which is likely to open up opportunities for the extreme levels of polyandry observed in this species ($> 80\%$; Calsbeek & Sinervo 2002b). In tree lizards (*Urosaurus ornatus*), habitat structure is a strong predictor of male polygyny vs. monogamy, with dense vegetation selecting for higher levels of polygamy (McLoskey *et al.* 1990). In painted dragons, however, males typically sit up and guard their territories from perch sites on the sand dunes facilitating territory patrol. In the majority of cases, this territory patrolling results in very low levels of mixed paternity.

Red males show signs of a more overtly aggressive strategy in the wild, such as beginning their territory defence

more than 3 weeks earlier than yellow males (at least in some years) and acquiring territories with higher perch densities, most likely at a metabolic cost reflected in their poorer body condition in the dry year. Nevertheless, this difference compared to yellow males does not seem to be significant enough to generate strongly divergent reproductive tactics, such as single-female mate guarding by red males vs. multiple-female, sneaking strategies of yellow males (and with no clearly discernible difference to orange males). What may explain this?

Elsewhere, we show a three times higher probability of paternity for yellow males in a sneaker role compared to red males in laboratory trials (M. Olsson, T. Schwartz, T. Uller & M. Healey, submitted). However, in free-ranging lizards, the level of multiple paternity is typically low (< 20% of clutches on average across years), and red and yellow males sire on average approximately equal numbers of offspring, suggesting that sneaking is simply never widely adopted. A possible explanation for this is that habitat heterogeneity is too low to allow each morph to exploit their optimal strategies, and we show that polyandry is significantly more common on territories where vigilance is relatively poorer. If this holds true, we would expect to currently see a higher frequency of the morph most facilitated by openness and aggressive patrolling (the red morph), which is exactly what we have seen during the last few years (Fig. 1). Interestingly, local year-to-year variation in habitat density and visibility in the Australian arid zone is largely driven by fire and hence, a natural cycling in these traits occurs and has done so for long periods of evolutionary time (Knox *et al.* 2006). A possible test of this scenario is to manipulate perch density or territory visibility as a driver of morph cyclicity and assess its response on the divergence in how males of the different morphs achieve their reproductive success.

Another factor that could contribute to variation in morph frequencies over time is a 'rare male advantage' effect if there is female choice (Andersson 1994). However, in painted dragons, we failed to find female choice for either colour in staged experiments in captivity (Healey *et al.* 2007). Nevertheless, our selection analysis shows a negative parameter estimate for red head colour in the year in which red dominates the population (57% red males). This effect may also contribute to the population-wide reduction in the red morph the following year (24% red males). However, this drastic decline in red frequency cannot be explained by sexual selection alone, since the absolute number of offspring generated by red males that year must be higher than for the other morphs taken together.

We conclude that the different male morphs of the painted dragon lizard show different behaviours in staged trials in congruence with a more dominant strategy (red), one of sneaking character (yellow), and one (orange) that did not deviate in reproductive success from the other two morphs but has not been further characterized. However, in the

wild, these differences do not bear out in overt differences in reproductive tactics. Given that c. 85% of siring success results in single-paternity clutches, and that polyandry occurs on less perch-dense territories, we suggest that habitat homogeneity may mask morph-specific behaviours and differences in how these males may maximize their siring success in the wild. Our study also offers a cautionary tale: studies of captive populations should be used carefully when making evolutionary inferences in the wild.

Acknowledgements

We thank E. Snaith and G. Snaith for logistic support, and the Australian Research Council (MO) and the Wenner-Gren Foundation (TU) for financial support. We are grateful to Dr Jon Slate for constructive criticism, which improved the quality of this manuscript.

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MO, MH, EW, NL and TU all have a broad interest in evolutionary biology (e.g., sexual selection and allocation, life history theory, behaviour, and evolutionary ecology and genetics). TS focuses on molecular genetics in evolutionary biology.

Supplementary material

The following supplementary material is available for this article:

Table S1 Number of alleles per locus, allele sizes, and frequencies for each of the 22 microsatellite loci, obtained from the genotypes of all of the 915 genotyped badgers.

This material is available as part of the online article from:
<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2007.03578.x>
 (This link will take you to the article abstract).

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