

Microsatellite determination of male reproductive success in a natural population of the territorial ornate dragon lizard, *Ctenophorus ornatus*

N. R. LEBAS*

Department of Zoology, University of Western Australia, Nedlands, Australia

Abstract

It is now evident that the genetic mating system can be very different to the observed mating system. However, it is less well known what makes particular individuals more (or less) successful than expected from the observed system. In this study the observed territorial structure of a field population of the agamid lizard, *Ctenophorus ornatus*, was compared with the mating system as evidenced by microsatellite parentage assignment. This study also investigated whether any male traits predicted reproductive success. Sixty-five per cent of clutches were sired at least partially by a male other than the main territory-holding male and 35% of clutches were sired by a male with no overlap of the female's territory. Multiple paternity was moderately frequent at 25% of clutches. Male chest patch size predicted territory size and the number of females in the territory, but did not predict reproductive success. Instead, male head depth and body size were independently related to the number of offspring sired. As male head depth also predicted the number of females in a territory, these males are likely to be gaining increased reproductive success as a consequence of the higher number of females in their territories. Larger body size males, however, did not have a greater number of females in their territory and instead had more extra-territorial copulations. Whether these extra-territorial copulations are due to female choice or success in male competition is unknown.

Keywords: lizard, mating success, microsatellites, parentage assignment, reproductive success, sexual selection, territories

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Introduction

It has now been widely shown that observed mating success does not necessarily equate to reproductive success determined using genetic markers (Hughes 1998). The greatest revelations about the differences between observed and genetic mating systems have been in socially monogamous birds, in which many species have been shown to engage in extra-pair copulations (Møller & Birkhead 1993). Genetic parentage assignments in polygynous species have found both high (Pemberton *et al.* 1992; Altmann *et al.* 1996; Abell 1997; Gullberg *et al.* 1997) and low (Amos *et al.* 1993; Coltman *et al.* 1999) correlations between the observed behavioural measure of mating success and

genetic reproductive success. Typically, there is low concordance in species with frequently changing dominance hierarchies and high concordance in species that form more stable hierarchies or in which a male guards a group of females (Coltman *et al.* 1999).

In polygynous territorial species, in which one male defends a territory encompassing several females, males that maintain the larger territories are those which are successful in intrasexual selection (Andersson 1994). In this situation it has usually been considered that females would not show mate choice as mating with the territory-holding male should ensure a high quality mate due to the male's success in intrasexual competition (Cox & LeBoeuf 1977), or if females did show choice it would be based on the same trait that enhanced male competitive abilities (Berglund *et al.* 1996). For example, in red-winged blackbirds, males that had the largest territories also had the most extra-pair copulations (Gibbs *et al.* 1990),

Correspondence: N. LeBas. *Present address: Environmental and Evolutionary Biology, University of St Andrews, St Andrews KY16 9TS, UK. Fax: 44 1334 463 600; E-mail: nl10@st-andrews.ac.uk

suggesting that females prefer males who also do well in male competition. However, recent studies contesting the generality of this finding have revealed that females do not always prefer the dominant male (Forsgren 1997; Qvarnstrom & Forsgren 1998). For instance, success in male competition may not be a reliable indicator of parental care or genetically determined viability (Qvarnstrom & Forsgren 1998).

A greater understanding of the discrepancies between the behavioural mating system and genetic mating system can be achieved by investigating which aspects of sexual selection are responsible for the differences. Sexual selection mediated through female choice for extra-pair copulations may be responsible for sexually dimorphic and dichromatic traits in socially monogamous species. In birds, a range of male traits has been found to predict a male's success in extra-pair copulations ranging from colour traits (Yezerinac & Weatherhead 1997) and body size (Whittingham & Lifjeld 1995) to song repertoire size (Hasselquist *et al.* 1996). Nevertheless, why females copulate with these extra-pair males as opposed to the territory holder is less well known. In the great reed warbler females had extra-pair copulations with males with larger song repertoires than their mate and offspring survival post-fledging was positively correlated with their father's song repertoire size (Hasselquist *et al.* 1996); evidence in favour of a good genes explanation for extra-pair copulations.

Lizards are an ideal species in which to examine the contrast between observed mating success and genetic reproductive success in a territorial polygynous species. In many territorial lizards the male territory encompasses the female's home ranges such that females do not seem to have the opportunity to assess or mate with other males (Stamps 1983). Consequently, it has been proposed, and the evidence supports, that in many territorial lizards male competition is the primary determinant of mating success (Stamps 1983; Olsson & Madsen 1998). For example, in a field study of the relative importance of inter- and intrasexual selection in the lizard, *Uta palmeri*, reproductive success was a consequence only of intrasexual selection (Hews 1990). Female choice of male traits has rarely been reported in lizards. In both cases in which female choice has been found the species were nonterritorial (Cooper & Vitt 1993; Censky 1997). Of the three species of lizards in which the genetic mating system has been examined, none are territorial (Abell 1997; Gullberg *et al.* 1997; Bull *et al.* 1998). However, in the striped plateau lizard, *Sceloporus virgatus*, in which males and females have overlapping home ranges, spatial proximity was a strong predictor of paternity (Abell 1997).

Ctenophorus ornatus is a sexually dimorphic and dichromatic agamid lizard (LeBas & Marshall 2000; LeBas 2000), which inhabits isolated granite outcrops in southern Western Australia. These discrete outcrops allow a complete

population to be studied and parentage assigned with a high probability that all adults in the population had been sampled. Females usually have discrete home ranges, have been observed to be aggressive to other females and appear to defend a proportion of their home range from other females (LeBas, in preparation). Males maintain territories exclusive of other males, but which typically encompass a number of female territories (LeBas, in preparation). As *C. ornatus* feeds on flying insects and females lay their eggs in burrows off the granite outcrop, it is unlikely that males are defending a resource which females require. Rather, males are likely to be defending the home ranges of a group of females in order to gain exclusive matings (Stamps 1983).

In this study, a field population of *C. ornatus* was followed over a breeding season and parentage assigned to the offspring using microsatellite markers. It was investigated whether male mating success estimated from territorial parameters was equivalent to realized reproductive success. Male traits were also examined for a relationship with territory size, territory quality and/or reproductive success. In addition, it was investigated whether male traits which predicted reproductive success were related to offspring viability as measured by offspring survival.

Materials and methods

Field site and methodology

This study was conducted at Tutanning Nature Reserve 200 km south-east of Perth, Western Australia (32°33' S, 117°20' E). A population of lizards was studied throughout the breeding season from mating to the end of hatching (October 1996 to April 1997). All the lizards on a 7200 m² outcrop were marked with two colour paint bands on the tail to facilitate identification during observations, and toe clipped for permanent identification and DNA collection. Lizards were collected from beneath exfoliated granite at first light for approximately four mornings per week, measured and returned to the site prior to daily emergence. Immediate processing prior to the time of first lizard activity ensured that territory maintenance by males was not influenced by the study process. The following variables were measured at each collection: snout vent length (SVL), mass, head width (across the middle of the tympanum) and head depth (over the middle of the tympanum). A photograph was taken of each lizard's ventral side and the chest patch area subsequently measured using Optimas Image Analysis software (Version 6.1). In females the presence of eggs was determined by palpation. The continuous collection regime resulted in recapturing most animals approximately every 2 weeks, as required to monitor female reproductive condition and approaching sexual maturity of juveniles.

A grid that encompassed the entire outcrop was marked at 5-m intervals for a 145 × 100 m area. Lizards were normally active during early morning and late afternoon and sought cover from the heat during the middle of the day. Lizards were observed during their periods of peak activity; typically 3 h in the morning and 3 h in the late afternoon. During observations lizard positions on the grid were recorded for the determination of territory size.

Females were allowed to lay naturally so that the population was disturbed as little as possible and to ensure that a true measure of reproductive success was obtained. Females become dusty when digging egg chambers; the day a female was first observed as dusty was recorded as her laying date. As the outcrop was observed continuously (typically 12 days of observations followed by 2 days away, repeated over the 6 months) offspring could be collected as soon as they hatched. This method was successful at collecting offspring immediately following hatching as the umbilical scar was not closed over in the majority of offspring at the time of collection (39 of 50).

Microsatellites and parentage assignment

Seven microsatellite markers developed for *Ctenophorus ornatus* (LeBas & Spencer 2000; LeBas submitted) were used to assign parentage to offspring. DNA was extracted from toe clips using the standard proteinase K phenol-chloroform procedure (Sambrook *et al.* 1989). Microsatellite markers were run according to the protocol of LeBas & Spencer (2000).

Parentage was assigned using CERVUS (Marshall *et al.* 1998). Parentage was assigned to each sex in a stepwise manner (Marshall *et al.* 1998). Offspring maternity was determined first, then paternity assigned with some offspring having a known parent. Simulations required for parentage assignment were run with the following parameters: 10 000 cycles; 12 potential parents (of each sex); 95% of candidate parents sampled; 93.7% of loci typed; 0.5% of loci mistyped. It was considered likely that virtually all candidate parents (95%) had been typed as the population was observed continuously from the onset of the breeding season and the discrete population allowed immediate detection of new arrivals. In addition, females cannot store sperm between breeding seasons eliminating the possibility that females fertilized eggs from matings in the preceding season (LeBas, unpublished data). The typing error rate was considered likely to be low (0.5%) as a random one-third of lizards were typed at least twice for each locus (no discrepancies were found) and audioradiographs were scored multiple times to eliminate reading errors.

One of the seven loci used to assign parentage had a high null allele frequency (null allele frequency = 0.086; LeBas & Spencer 2000). Null alleles can cause the false exclusion of true parents due to incorrect typing of a

heterozygote as a homozygote. As this locus was highly polymorphic it was desirable not to exclude it from the analysis. Instead, to prevent false exclusion, all offspring which were homozygotes were entered as typed at only one allele. Therefore, offspring carrying their parents' null allele would not mismatch with their true parent.

Related potential parents can confound parentage assignment (Thompson & Meagher 1987). CERVUS, however, is robust to the confounding effects of most types of relatives with only small over-estimations of confidence in most scenarios (Marshall *et al.* 1998). The exception is when full-sibs of the offspring are also considered as candidate parents (Marshall *et al.* 1998). In this, usually rarer, situation, confidence can be significantly overestimated (Marshall *et al.* 1998). While it cannot be discerned if these types of relatives are a problem in this study, their presence cannot be ruled out. However, for 48 of 88 assignments (55%) there were no other potential parents which did not mismatch at at least one locus. In addition, parentage was assigned at the highest confidence level (95%). As field parentage is often assigned with 80% confidence (Coltman *et al.* 1998; Marshall *et al.* 1998) the confidence levels used here can withstand some overestimation.

Territory size

Lizard territory sizes were determined using the minimum convex polygon method with 95% edges and adjusted arithmetic mean (Ranges V; Institute Terrestrial Ecology). Territories of males moved or changed size with variation in the number of males in the population. Males whose territories changed as a consequence of the death or arrival of another male had territories calculated before and after the change to prevent inflated territory sizes due to territory shifts. Territory size for each male was thus an average of all the territories he held during the season. Males whose home range extended over the majority of the outcrop were considered not to be territory holders, as they were moving over the whole of the outcrop rather than defending a certain area. These males had a territory size of zero in the analyses. Females showed a similar pattern to males in that not all females had discrete home ranges and throughout the breeding season there were shifts in territory size and position. As females can lay up to three clutches in a season, female territory size was calculated separately for each clutch.

Two measures of territory quality were determined. First, the area of shelter sites within a territory and second, the area of high rocks in the territory which are typically utilized as lookouts and display platforms (LeBas, personal observation). For each grid the length and breadth of the exfoliated granite sheets and any rocks under which the lizards could hide were measured. For rocks over 10 cm high, the height of the rock was also measured.

For each grid the area of cover and the area of rocks over 10 cm were also calculated. The area of cover in a territory was defined as the area of exfoliated granite in each territory and represents the amount of shelter that was available to the territory holder. The area of look-outs was the total area of rocks over 10 cm which could be used to monitor territory infringements and to display territory ownership.

A female's territory-holding male was defined as the male which overlapped her territory the greatest (overlap analyses, Ranges V). As a consequence of male death or territory take-over there were cases in which two males overlapped the female's territory by >50%. In these cases both males were considered the main territory holder and an average of their traits used in analysis.

Male trait relations

Four male traits were examined in this study: male SVL, head depth, head width and chest patch area. However, these traits were correlated with one another such that there was a significant collinearity problem when they were all entered in a multiple regression (SVL: tolerance = 0.04; head width: tolerance = 0.03). To eliminate collinearity problems and reduce the number of variables, the traits were entered into a principal components analysis. The components were rotated using the Varimax method with Kaiser normalization (SPSS Inc.). Three principal components were extracted and used in analyses.

Photographs were not obtained from two males and therefore chest patch area could not be measured for these males. As chest patch area was entered into the principal components analysis, all the factors which were extracted were missing values for these two males. In cases in which traits other than chest patch were found to have a significant effect in the multiple regression the results were checked in a linear regression with the full sample size. In analyses that included only males which sired offspring, the reduction in sample size was too great to use the principal component scores and instead the variable that had the highest loading in the principal component in question was used in the analysis.

Correlates of reproductive success

While the total number of offspring an individual has is a direct measure of fitness for that breeding season, it is biased against males that reached sexual maturity late in the breeding season and only had the potential to fertilize a small proportion of the eggs for the season. In addition, some large males disappeared during the breeding season and presumably died. As *C. ornatus* can live up to 5 years in the field (Bradshaw 1965), males in the 'middle' age group are likely to have higher reproductive success

in any one breeding season simply as a consequence of their age at that time. As one purpose of this study was to examine the relationships between male traits and reproductive success it was necessary to control for the variation in reproductive success which was likely to be directly attributable to age. While age has been shown to be an important factor in sexual selection (Clutton-Brock 1988), large sample sizes of each age group or monitoring over time is required to examine these effects. These requirements were not feasible in this study.

To account for the age/maturity variation this study examined the number of offspring a male had, taking into account the number he could have sired given the time he was present and sexually mature on the outcrop. To calculate this measure first, the average hatching clutch size was determined by dividing the total number of offspring that hatched by the number of clutches these offspring were from. This gave an average hatching clutch size of 2.14 offspring. Females can lay between one and three clutches and typically lay each round of clutches at around the same time. For each clutch the number of males that were present on the outcrop and sexually mature in the interval between when a female laid her previous clutch and the laying date of the clutch in question was determined. For the first clutch laid the time interval was from the beginning of the season to the date of laying. Males present during this time interval were considered to have had the opportunity to sire the clutch. Next, the average number of offspring per clutch was divided by the number of males available (according to the methods above) to sire that clutch. This process was repeated for each clutch and these values summed for each male to give the total number of hatched offspring a male had the potential to sire. The actual number of offspring a male sired was divided by this potential number of offspring. This measure is referred to as a male's realized number of offspring.

Two other measures of male reproductive success were examined. First, the number of extra-territorial offspring a male sired and second, the average number of offspring a male sired in a clutch. For the first measure offspring were considered to be from extra-territorial copulations if the male in question was not the territory holder for that female. The number of offspring from extra-territorial copulations was adjusted for the potential number of offspring a male could have sired as above. For the second measure the total number of offspring a male sired was divided by the number of clutches these offspring were from to give an average sired clutch size.

Male realized reproductive success was not normally distributed, however, the standardized residuals from the regression on male principal component scores were normal and so parametric statistics were used (Zar 1984). All other traits examined were normally distributed.

Offspring viability

Mean offspring survival to 15 weeks, as a measure of offspring viability, was examined for a relationship with male traits which predicted reproductive success. Survival was measured as the number of weeks an offspring lived after hatching. Data were censored at 15 weeks as at this age the size of a fast growing offspring was equivalent to the smallest lizard which arrived at the outcrop during the study. Therefore, 15 weeks was considered the age at which offspring have the potential to disperse, consequently after this time offspring absence on the outcrop cannot be attributed to death. For each male the proportion of his offspring that survived to 15 weeks was calculated. As proportions typically have a binomial rather than normal distribution this measure was arcsin square-root transformed (Zar 1984).

Results

Parentage assignment

Parentage was assigned for both parents to 43 of the 50 offspring with 95% confidence. An additional offspring was assigned to a female with 80% confidence. As all other potential female parents had a mismatching allele with this offspring, the female with 80% confidence was considered to be the true parent. This assignment allowed paternity to be determined with 95% confidence. This offspring was included in all subsequent analyses; thus there were 44 offspring with both parents known. For one of the unassigned offspring, maternity was assigned with 95% confidence, but the father could not be identified.

Multiple paternity was detected in five of 20 clutches (25%). Females produced offspring sired by a male other than, or in addition to, the main territory holder in 13 of 20 clutches (65%). Thus only 35% of clutches were sired solely by the territory-holding male. A number of females' territories were overlapped slightly by a male other than the male classified as the territory holder. Six of the 13 clutches sired by external males were sired by these 'overlapping' males. Seven clutches (35%) were sired by a male that was never recorded within a female's territory.

Two of 50 offspring (4%) were fathered by males that were no longer in the population (due to death or dispersal) from the time a female laid her previous clutch to the time she laid the clutch of the offspring in question. This suggests that females can store sperm between clutches, although 4% of offspring sired in this manner is small enough to be within the realms of false parentage assignment (5%).

Observed vs. genetic mating system

A male's territory size was positively related to the number of females in the territory (Spearman rank correlation:

Table 1 Relationships between male traits. Pearson and partial correlations

Trait	N	Correlation coefficient	P
Chest patch area vs. SVL	10	0.61	0.06
Head width vs. SVL	12	0.96	0.001
Head depth vs. SVL	12	0.73	0.01
Chest patch area vs. head depth*	7	0.55	0.13
Chest patch area vs. head width*	7	0.25	0.52
Head depth vs. head width*	9	0.75	0.02

*Partial correlation controlling for snout vent length (SVL).

Table 2 Principal component factor loadings of male traits. Traits which contribute the greatest to the factor are given in bold. Varimax rotation method with Kaiser normalization

Trait	PC1	PC2	PC3
Chest patch area	0.291	0.908	0.301
SVL	0.920	0.286	0.259
Head width	0.843	0.282	0.442
Head depth	0.437	0.409	0.801
% of variance explained	45.84	28.85	24.87

$N = 11$, $Rho = 0.78$, $P = 0.005$). However, the number of females a male had in his territory (the observed measure of reproductive success), did not predict his realized reproductive success, although it was in the same direction (least squared regression: $F_{1,10} = 3.93$, $b = 1.28 \pm 0.644$, $r^2 = 0.23$, $P = 0.08$).

Male trait relations

All traits other than chest patch area were significantly correlated with snout vent length (SVL; Table 1). In a partial correlation controlling for SVL there were no relationships between the head measurements and chest patch area, but head depth and head width were significantly correlated (Table 1). Principal components analysis reduced the four traits to three independent principal components (PC) which together explained 99.55% of the variance (Table 2). The first principal component was mostly weighted by body size (PC1), the second by chest patch area (PC2) and the third by head depth (PC3, Table 2). Note that whilst head depth and head width were significantly correlated, in the principal components analysis head width weights the first PC and head depth the third PC. Thus, there is no 'head size' principal component and thus these two head measures explain variation in head depth and width separately.

Table 3 Three multiple regression models. The first model is the relationship between a male's territory size and principal components (PC) 1–3 (whole model: $F_{3,5} = 9.75$, $P = 0.016$). The second model is the relationship between the number of females in a male's territory and PC 1–3 (whole model: $F_{3,5} = 6.79$, $P = 0.03$). The third model is the relationship between a male's realized number of offspring and PC 1–3 (whole model: $F_{3,5} = 13.29$, $P = 0.008$)

Trait	Coefficient	SE	<i>t</i>	<i>P</i>
Territory size				
PC1 (body size)	-1.178E-02	0.008	-1.484	0.198
PC2 (chest patch area)	4.387E-02	0.009	4.902	0.004
PC3 (head depth)	1.491E-02	0.008	1.871	0.120
Number of females in territory				
PC1 (body size)	0.113	0.554	0.203	0.847
PC2 (chest patch area)	2.307	0.624	3.695	0.014
PC3 (head depth)	1.523	0.556	2.740	0.041
Reproductive success				
PC1 (body size)	0.800	0.187	4.278	0.008
PC2 (chest patch area)	0.334	0.211	1.584	0.174
PC3 (head depth)	0.837	0.188	4.460	0.007

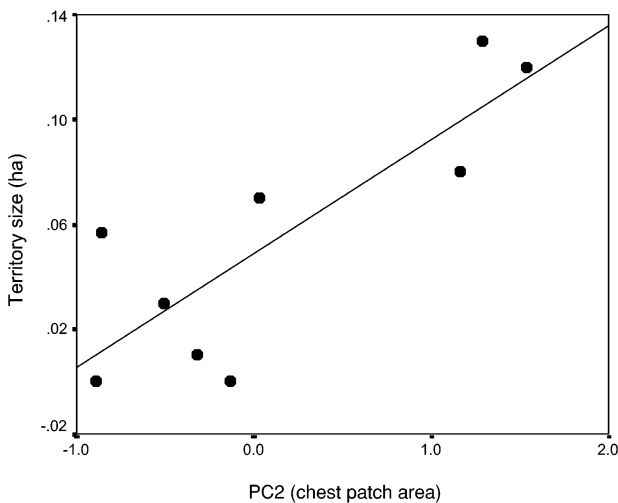


Fig. 1 Relationship between male PC2 (chest patch area) and territory size.

Correlates of territory size

Territory size was strongly positively correlated with the area of cover in the territory (Pearson correlation: $n = 11$, $r = 0.98$, $P < 0.001$) and the area of lookouts (Pearson correlation: $n = 11$, $r = 0.93$, $P < 0.001$). Therefore, only territory size was examined further as this measure explained so much of the variation in territory quality. In a multiple regression analysis PC2 (chest patch) predicted male territory size (Table 3). This relationship between PC2 and territory size was confirmed in a linear regression (least square regression: $F_{1,7} = 15.36$, $b = 4.34E-02 \pm 0.011$, $r^2 = 0.64$, $P = 0.006$; Fig. 1). This relationship is also maintained when only males that have a territory size greater than zero are examined (least square regression: $F_{1,5} = 11.49$, $b = 3.76E-02 \pm$

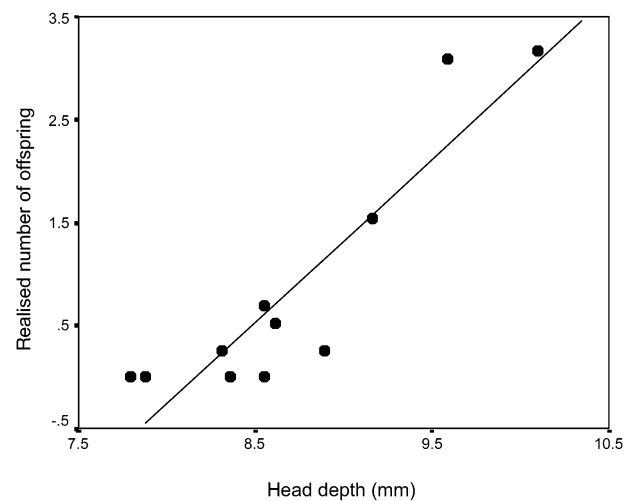


Fig. 2 Relationship between male head depth and realized reproductive success (number of offspring sired divided by the potential number of offspring that the male could have sired).

0.011 , $r^2 = 0.64$, $P = 0.019$). The number of females in a male's territory was significantly related to both male PC2 (chest patch) and PC3 (head depth) (Table 1). Thus male chest patch area predicts territory size and the number of females in the territory, whereas male head depth predicts only the number of females in the territory.

Correlates of reproductive success

Both PC1 (body size) and PC3 (head depth) significantly predicted male reproductive success (Table 3). The relationship between male head depth and reproductive success was confirmed in a linear regression using the full sample size available (Spearman rank correlation: $N = 11$, $r = 0.848$, $P = 0.001$; Fig. 2).

The higher reproductive success for males with larger head depths is likely to be a consequence of the greater number of females in these males' territories. The higher reproductive success of large body size males, however, cannot be explained by a greater number of females in their territories. Larger body size males may gain enhanced reproductive success either by having a greater number of offspring per clutch that they sire or obtaining more extra-territorial copulations. There was no relationship between male SVL and the average number of offspring a male sired in a clutch (least squared regression: $F_{1,5} = 2.56$, $b = 4.42E-02 \pm 0.028$, $r^2 = 0.21$, $P = 0.17$). However, male SVL predicted the number of realized extra-territorial offspring he sired (least squared regression: $F_{1,5} = 14.59$, $b = 0.112 \pm 0.029$, $r^2 = 0.69$, $P = 0.01$).

In addition, when females had offspring sired by a male other than the territory-holding male, the PC1 (body size) of the sire of a female's clutch was larger (mean = 0.431 ± 0.2111) than that of her territory holder (mean = 0.0085 ± 0.1713 ; paired t -test d.f. = 11, $t = 2.81$, $P = 0.02$). The sample size for this analysis is reduced due to the missing chest patch areas for two males. Analysis with SVL instead of PC1 confirmed the above result; the sire of a female's clutch had a larger SVL (mean = 81.11 ± 1.045) than the territory-holding male (mean = 78.04 ± 0.997 ; paired t -test d.f. = 17, $t = 2.72$, $P = 0.01$).

There was no relationship between the number of offspring a male sired within his territory and the number of offspring he sired as a consequence of extra-territorial copulations (Pearson correlation: $N = 7$, $r = 0.60$, $P = 0.15$). Therefore, males that are successful within their territory are not necessarily successful at gaining extra-territorial copulations.

Offspring viability

Male SVL did not reflect male quality as measured by the proportion of his offspring which survived to 15 weeks (least squared regression: $F_{1,5} = 0.98$, $b = 2.02E-02 \pm 0.020$, $r^2 = 0.16$, $P = 0.37$). Neither was head depth related to the proportion of offspring that survived to 15 weeks (least squared regression: $F_{1,5} = 0.81$, $b = 8.89E-02 \pm 0.209$, $r^2 = 0.04$, $P = 0.69$).

Sexual dimorphism

The sexual dimorphism in head depth (log transformed) was analysed using an ANCOVA with sex as a main effect and SVL (log transformed) as a covariate. The interaction term was not significant and was removed from the model (whole model: $F_{2,27} = 34.53$, $P < 0.0001$, sex $F_{1,27} = 7.182$, $P = 0.012$, SVL $F_{1,27} = 68.59$, $P < 0.0001$). Therefore, the allometry of head depth is the same for both sexes. Males had deeper heads than females controlling for SVL (LS

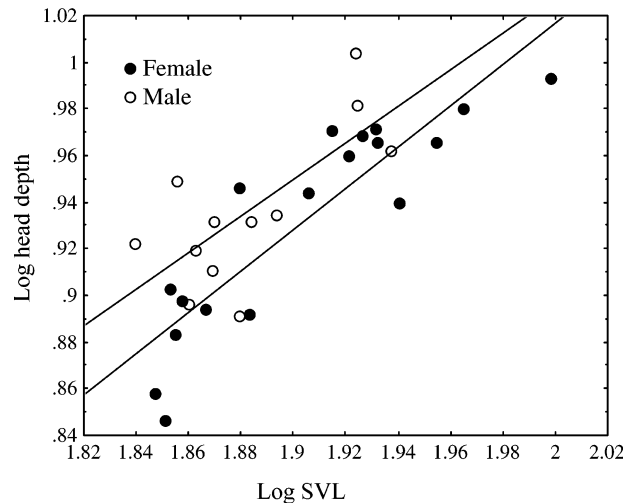


Fig. 3 Relationship between head depth and snout vent length (SVL) for males and females. Males have significantly deeper heads than females.

mean \pm SE, males = 0.948 ± 0.0065 , females = 0.925 ± 0.0052 ; Fig. 3). There was no significant difference in SVL between males (mean = 76.56 ± 1.59) and females (mean = 80.67 ± 2.01 ; t -test, d.f. = 28, $t = 1.47$, $P = 0.15$).

Discussion

This study revealed that observed measures of mating success were not significantly related to genetic reproductive success in a polygynous territorial lizard. This study also showed that traits which predicted male territory attributes, and hence were likely to be important in male competition, did not always predict reproductive success. Chest patch size determined male territory size and number of females in the territory, however, it was unrelated to reproductive success. Head depth predicted the number of females in the territory and reproductive success. Furthermore, although male body size was not related to any of the territory variables, it was a significant predictor of reproductive success. These larger body size males appear to gain higher reproductive success through extra-territorial copulations. Therefore, chest patch area and head depth are important in male competition over territories, but only head depth and body size appear to be currently under directional sexual selection in this population.

Multiple paternity and sperm storage

Twenty-four per cent of *Ctenophorus ornatus* clutches showed multiple paternity. As average clutch size in *C. ornatus* is small (typically two eggs hatch) multiple paternity, as determined by paternity of offspring, is likely to be a conservative estimate of multiple mating. Multiple paternity

has been documented in all species of lizards which have been examined; these have included a diverse array of mating systems (Olsson & Madsen 1998). Multiple paternity has been detected in the primarily monogamous sleepy lizard, *Tiliqua rugosa* (Bull *et al.* 1998), in the majority of clutches in the highly promiscuous sand lizard, *Lacerta agilis* (Gullberg *et al.* 1997) and in the polygynous, but nonterritorial, striped plateau lizard, *Sceloporus virgatus* (Abell 1997). Multiple paternity over such an array of mating systems, together with the findings from this study on a territorial species, suggest that sperm competition may be a common feature of lizard mating systems (Olsson & Madsen 1998).

There was also some indication that females store sperm between clutches. Two males that were no longer being observed in the population (due to death or dispersal) from the time a female laid her previous clutch to the time she laid her next clutch were assigned paternity of offspring from the second clutch. However, the frequency with which this was found, 4%, is sufficiently small to be within the error of parentage assignment. Sperm storage is common in reptiles, with some species storing sperm for up to 7 years (Birkhead & Møller 1993). However, as female *C. ornatus* cannot store sperm between breeding seasons (LeBas, unpublished data) and the low incidence of sperm storage in this field study suggests it is unlikely that sperm storage is important in this mating system.

Observed vs. genetic mating system

The number of females a male had in his territory did not predict his realized reproductive success, although there was a trend. Instead, male reproductive success was predicted by two principal components which represented a male's body size and head depth. Therefore, females do not exclusively mate with their territory-holding male. Two-thirds of the clutches laid were sired at least partially by a male other than the territory-holding male and 35% were sired by a male whose territory had never overlapped the females. Consequently, a measure of reproductive success in this species which relied on the number of females within a male's territory would only provide an approximation of the true genetic reproductive success. Furthermore, this observational measure of reproductive success would miss selection on traits such as male body size which did not predict territory size.

Male chest patch size

Males with larger chest patches had larger territories and consequently had access to a greater number of females. In *C. ornatus* chest patch size is dependent on condition (LeBas 2000). The chest patch is emphasized during the push-up displays that males perform to one another

and hence may function as a signal of a male's condition and thus fighting ability. In sand lizards, *L. agilis*, the area of green on a male's flank is positively correlated with male condition and a larger badge of green was indicative of males with higher fighting ability (Olsson 1994). Similarly, male tree lizards, *Urosaurus ornatus*, with a larger blue spot on their dewlap won more contests (Thompson & Moore 1991). Male chest patch size in *C. ornatus* may settle territorial disputes without the costs of fighting.

It is expected that when traits reliably signal male quality and/or have been tested through male competition females should also prefer these traits (Cox & LeBoeuf 1977; Kodric-Brown & Brown 1984; Berglund *et al.* 1996). Female preference for dominant males has been found widely (Andersson 1994) as has female preference based on the same traits as those used in male competition (Berglund *et al.* 1996). However, in species with multiple traits, different traits may be used in different contexts. For example, in the scarlet-tufted malachite sunbird, *Nectarinia johnstoni*, pectoral tufts are involved in territorial defence and tail length in mate choice (Evans & Hatchwell 1992a,b).

In laboratory mate choice trials female *C. ornatus* showed no preference for males on the basis of chest patch size (LeBas 2000). This field study confirms that result; male chest patch size was not related to male reproductive success. In addition, females do not move their territories when male territories changes (LeBas, in preparation), further suggesting that the association between a female and her territory-holding male is not a consequence of female choice for that male. Similarly, in the iguanid lizard, *U. palmeri*, females alter their home ranges in relation to food, not male distribution (Hews 1993). Therefore, in *C. ornatus*, a female's territory-holding male is likely to have been determined by male competition rather than female choice for that male. It may be a *C. ornatus* female's best option to mate with the territory-holding male as this male has proven his quality in male competition (Cox & LeBoeuf 1977; Berglund *et al.* 1996). However, if females have no control over which male guards her territory and her territory-holding male happens to be of poor quality, then it may favour females to mate with a male other than her territory holder.

Male head depth

Male head size has been found to be subject to sexual selection in a number of lizard species (Olsson & Madsen 1998). In *C. ornatus* male head depth may be an advantage in intrasexual selection as male lizards bite one another while fighting. In *U. palmeri* males with greater head depths were more successful in male contests (Hews 1990) as were sand lizards, *L. agilis* (Olsson 1992), skinks, *Eumeces laticeps* (Vitt & Cooper 1985) and numerous teiid

lizards (Anderson & Vitt 1990). In the sleepy lizard, *T. rugosa*, small males that were paired with a female had significantly broader heads than unpaired males, a result which could be due to male competition (Bull & Pamula 1996). Males with larger heads are likely to have greater jaw musculature which may reflect fighting ability. Indeed, in the skink *E. laticeps*, male head depth increases during the breeding season (Vitt & Cooper 1985). In *C. ornatus* male head depth may be related directly to male fighting ability and be important in conflicts which escalate beyond signals such as chest patch size. A deeper head may allow a male to defend a greater number of females. Furthermore, males with deeper heads may be better able to defend females within their territory, ensuring paternity more often than males with large chest patches but smaller head depths.

Male body size and reproductive success

Male body size was not related to male territory size, but strongly predicted male reproductive success. Larger body size males have a higher reproductive success that is not due to siring offspring with females within their territory. This enhanced reproductive success was not due to larger body size males having larger clutch sizes or siring more of a clutch in multiple paternity clutches. Rather, larger body size males sired a greater number of extra-territorial offspring. In accordance with this finding the sire of a female's clutch was larger than that of her territory-holding male. These results could be explained in two ways, first by male competition in which larger body size males succeed in gaining access to other male's territories. Second, by female's visiting other male's territories and exercising mate choice.

Male body size determines male mating success in at least 17 species of lizards (Olsson & Madsen 1998). This is typically a consequence of larger males winning in male contests and hence gaining greater access to females (Anderson & Vitt 1990; Olsson & Madsen 1998). This study differs in that larger males do not have bigger territories or more females within their territories, but rather have higher reproductive success due to extra-territorial copulations. The same principals, however, may apply, larger body size males may gain access to females in other males territories through success in contests.

Alternatively, the high reproductive success of larger body size males may be due to female choice for these males. In birds it has been shown that males successful in extra-pair copulations confer genetic benefits to the offspring (Kempenaers *et al.* 1992; Hasselquist *et al.* 1996). As there are no material benefits to mating or parental care in *C. ornatus*, females are also likely to be seeking genetic benefits in their choice. However, offspring sired by larger body size males did not have increased survival,

although the small sample sizes temper this conclusion. In choice trials, females do not prefer larger males (LeBas 2000), which suggests females are not choosing larger body size males. However, spatial association was used as the measure of choice in the laboratory study. In the field population larger body size males are gaining their enhanced reproductive success through extra-territorial copulations. Females territories do not change when male territories change and hence females may show no preference for the male in whose territory they reside (LeBas, in preparation). However, during receptivity females may visit a male that they wish to sire their offspring. Thus, while female choice of larger body size males appears unlikely, it cannot be ruled out for this population.

Sexual dimorphism

This study documents that in the *C. ornatus* population studied there are multiple male traits that are subject to sexual selection. An observational study on *C. ornatus* would have likely concluded that males with large chest patch areas had high reproductive success. While the evolution of the sexual dichromatism in chest patch suggests such an advantage, in this study chest patch area was unrelated to reproductive success. Head depth is sexually dimorphic in *C. ornatus* and as this study shows is currently under directional sexual selection. The extreme dorsal flattening in *C. ornatus* gives this species a head shape distinctly flatter than other members of the genus (Bradshaw 1965). This adaptation to sheltering under exfoliated granite means that head depth may be under opposing natural selection as well. Snout vent length was not found to be sexually dimorphic in the population studied here, although data from a larger museum sample suggests that males are larger (LeBas, unpublished data). However, selective collecting in the museum sample cannot be dismissed. Sexual size dimorphism is a common feature in lizards (Stamps 1977).

The genetic mating system of *C. ornatus* in the study population appears to be one in which male territoriality is not an accurate indicator of male reproductive success. This variance arises from a higher reproductive success for large body size and deeper head depth males, but not for males with large chest patches. Whether the selection on male body size is a consequence of greater access to females derived through fighting or female choice is not clear.

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- This study was part of a PhD thesis by Natasha LeBas, in which she examined sexual selection and the mating system of the ornate dragon lizard, *Ctenophorus ornatus*. Her research interests include lizard mating systems, the role of colour in mate choice and molecular parentage determination.
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