Unexpected high fitness payoff of subordinate social tactics in male collared lizards

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Sexual selection theory often predicts that dominant males will sire more offspring than males displaying subordinate social tactics. We combined the records of space use by collared lizard, Crotaphytus collaris, females and records of social and spatial behaviour of males displaying two markedly different social tactics (territorial and nonterritorial) with genetic determination of parentage to test how variation in male social tactics influences the distribution of reproductive success. In marked contrast with predictions based on their social and spatial behaviour, territorial males did not monopolize maternity of offspring with the females that they defended, and on average, nonterritorial males obtained reproductive success equal to that of territorial males, both within and among successive clutches. When all males were analysed together, none of the traits that are often hypothesized to promote fitness in other lizards were under strong sexual selection in collared lizard males. Among territory owners alone, however, there was positive directional selection on body size. Both the unexpected success of non-territorial males and exceptionally high levels of multiple paternity appear linked in part to features of the habitat at our study site that diminish the ecological potential for territorial males to monopolize mating opportunities with females that reside in their defended areas. If female collared lizards derive any fitness benefits by mating with multiple males, it is likely that these are genetic rather than material.

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determining parentage assignments accurately is accentuated by mounting evidence that females choose to mate with and/or select sperm from multiple males, even in species where males judged to have to high RHP monopolize females socially (Gibbs et al., 1990; Hughes, 1998; Olsson, Madsen, Shine, Gullberg, & Tegelström, 1994). By mating with multiple males, females may gain increased paternal care, access to resources required for reproduction, or increased quantities of sperm to ensure fertilization of all eggs (Andersson & Simmons, 2006; Slatyer, Jennions, & Blackwell, 2012). Alternatively, by mating with multiple males, females may derive genetic benefits that promote the survivorship and/or attractiveness of their offspring (Byrne & Keogh, 2009; Kokko, Jennions, & Brooks, 2006; Kuiper, Pen, & Weissing, 2012).

Genetic determination of parentage coupled with observations of social interactions among all potential parents, provides a much more accurate estimate of mating relationships and the distribution of reproductive success, especially in social systems where individuals use more than one reproductive tactic (Double & Cockburn, 2003; Zamudio & Sinervo, 2003). The necessity of combining genetic and behavioural measures is accentuated by studies demonstrating marked discrepancies between mating relationships established using genetic techniques versus those estimated using behavioural observations (Gibbs et al., 1990; Hughes, 1998; LeBas, 2001).

We combined genetic determination of parentage with detailed observation of the social and spatial behaviour of individual collared lizards to test whether defence of territories by high RHP males promotes monopolization of females, or whether females mate with multiple males, including those that do not defend territories. Higher RHP and prolonged, frequent courtship predict that territorial males should garner a reproductive advantage over mature, but nonterritorial, males (Baird et al., 1996; Lappin & Husak, 2005), and that females should preferentially mate with the males that defend areas overlapping their home ranges (Baird, 2013a; Baird, Fox, & McCoy, 1997; Baird et al., 2007). In our study population, however, the possibility that spatial overlap and courtship frequency do not accurately predict mating relationships may be especially high. Female home ranges are partially overlapped by up to three territorial males and numerous nonterritorial males (Baird et al., 1996). Moreover, because the habitat consists of continuous boulder fields having nearly unlimited crevices (Baird & Sloan, 2003), nonterritorial intruders can readily hide to avoid attacks by territory owners without leaving the vicinity of female home ranges. Increased access to females while being able to avoid aggression may promote opportunities for mate choice and multiple mating by females.

METHODS

Study Population

We conducted this study during 20 March–31 October in 2007 and 2008 at the Arcadia Lake Dam flood control spillway located 9.6 km east of Edmond, Oklahoma Co., OK, U.S.A. (Baird, Timanus, & Sloan, 2003). Collared lizards at Arcadia Lake occupy three topographically homogeneous patches of boulders (1230–19,853 m²) used to construct flood-control channels (Curtis & Baird, 2008). This study site is well suited for documentation of behavioural interactions among individuals because human access is restricted, lizards are undisturbed, the homogeneity of rock patches allows prolonged and unobstructed observation, and rock patches are mapped to scale using GIS measurements (accurate to ±1.0 m) of markers arranged in 10 m grids (Baird & Timanus, 1998; Baird et al., 2003). All lizards at this study site are noosed as hatchlings and marked (the terminal phalanges of three digits are clipped for permanent identification, and unique combinations of nontoxic acrylic paint spots are applied to the dorsum for identification of individuals from a distance; see Ethical Note below). The ages of all lizards used in the present study were known because they were periodically recaptured for marking and measurement since their first capture as hatchlings.

Previous studies on social and spatial behaviour have shown that females maintain strong philopatry to small nondefended home ranges where they spend most of their time scanning for arthropod prey from elevated perches (Baird et al., 1996; Baird & Sloan, 2003). Males typically acquire territories at the beginning of their second season. Territorial males rely on high rates of patrol and broadcast display, punctuated by occasional chases and fights, to advertise and defend territories (Baird, 2013a). Territories of males partially or completely overlap the home ranges of up to eight females, with whom the males frequently interact during prolonged (up to 30 min) courtship encounters throughout the reproductive season (Baird, Sloan, & Timanus, 2001; Baird et al., 2007). Even though males are sexually mature during their first year, they typically adopt inconspicuous subordinate social tactics characterized by low patrol and display rates. When detected by territory owners, nonterritorial males flee and hide in crevices, which are abundant at Arcadia Lake (Baird & Sloan, 2003). Nevertheless, first-year males interact with females when territory owners have not detected them (Baird et al., 2003).

Recording Spatial and Social Data

During the reproductive season (1 May–15 July), one of the authors recorded mapped census sightings and focal individual observations (both described below) to document the spatial and social behaviour of lizards in the Arcadia Lake population. For the present study, we recorded both types of data on all mature males (N = 27), and census data on all females (N = 28). Data were recorded on scale-drawn maps when the substrate temperature was 30–38 °C, over which collared lizard activity is independent of substrate temperature (Baird et al., 2001). We recorded behavioural data (described below) from 1 May to 30 June, when females produce up to three successive clutches (see Schedule of Egg Production and Hatching below; Baird et al., 2001).

Censuses of the entire study site (N = 30; 15 censuses during May, 15 censuses during June) involved recording the point locations and identities of all emergent lizards on scale-drawn maps. Census sightings for males were combined with the beginning and ending points of focal traces (described below) to construct maps of territories and home ranges using the minimum convex polygon technique (Turner, 1971). The number of points used to construct the composite maps of male territories (N = 60–65), as well as female home ranges (N = 30–40), equaled or exceeded the number necessary to achieve an asymptotic relationship when home range/territory area was graphed against the number of sightings (Baird & Sloan, 2003; Stone & Baird, 2002), following the method of Rose (1982).

Focal observations (sensu Altman, 1974) involved tracing the path of travel and recording all of the social acts initiated by subject males on scale-drawn maps (Baird, 2013a). We recorded 20 min focal observations (N = 10/male) on different days throughout the reproductive season. Male collared lizard activity does not vary as a function of time of day from 0900 to 1300 hours when we recorded focal observations (Baird et al., 2001). However, to control for any possible temporal bias, each day we observed individual males that were present on the site in random order.

Social behaviour is initiated by male collared lizards in two distinct contexts. Displays that are broadcast when males are on elevated perches at least 5 m from conspecifics are the most
common (Baird, 2013a; Baird & Curtis, 2010). Most broadcast displays involve males extending all four legs to elevate the torso, which is compressed laterally while the dewlap is extended (see photographs in Baird, 2013a, 2013b). While holding this fullshow posture, males almost always flex their legs to raise and lower the head and torso 1–12 times (=pushups) in succession. Much less frequently (2%) males display by walking in a circular or figure-eight pattern while remaining on a single perch (Baird, 2013a, 2013b; Baird & Curtis, 2010).

In contrast to broadcast displays, proximal aggressive encounters with male rivals (=contests) involve one male running to within 1 m of a male rival, followed by escalation into one or more of the following: an exchange of displays (fullshows, pushups) while the two males remain in close proximity (1 m), the recipient fleeing, reciprocal chases (up to 40 m) back and forth, and occasionally attacks involving wrestling and biting (Baird, 2013a, 2013b). Because broadcast displays are given when males pause on perches and are distant from all conspecifics, they are readily distinguished from contests when males are chasing, chasing or fighting a rival, or when males are displaying to a rival in close (1 m) proximity that is reciprocating by also giving displays.

Males also initiate proximal encounters with females that function in both a courtship (Baird, 2013a; Baird & Curtis, 2010; Baird et al., 2007). Courtship encounters involve the same displays that males broadcast from a distance (described above), but these are also given when one male and female are within one body length of one another, and when both lizards are making frequent and prolonged physical contact (Baird, 2004). Physical contact includes one lizard mounting and sitting on the dorsal surface of their partner, superimposition of the legs and/or tails, nudging their partner with the snout (Baird, 2013b), or simply perching adjacent while touching the other lizard (Baird, 2004; Baird & Sloan, 2003). Males sometimes grasp the dorsal skin of the female’s neck and attempt to juxtapose their vent with that of the female, presumably to attempt copulation. However, because attempts at copulation usually result in both lizards moving into a refuge (Baird et al., 2003), we could not reliably determine whether or not mounting resulted in successful intromission.

Using the cumulative focal observations recorded on each male, we calculated hourly frequencies of broadcast displays, contests between rival males and courtship encounters with females (separately) by dividing the total number of these acts/events by the total focal observation time (Baird et al., 2007). We measured mapped traces using a digital planimeter (Planixx, 2000) and calculated the hourly rate of patrol by dividing the total distance travelled by the cumulative observation time on individual males (Baird et al., 2007).

**Schedule of Egg Production and Hatching**

Adult lizards typically emerge from hibernacula from late March to early April and remain reproductively active until 15 July (Baird et al., 2001). In 2007, we documented the schedule of egg development and oviposition by capturing all females (N = 28) present on the site every 7–10 days to weigh and measure them, and palpate their abdomens. At each palpation, we characterized follicular/egg development using the egg size and shape criteria developed for females in this population (Baird, 2004; Telemeco & Baird, 2011). The abdomen becomes increasingly swollen as females ripen their eggs, whereas oviposition is marked by pronounced loss in body mass (30–50% of total mass) and mud caked on the toes and integument from digging nests (Baird, 2004). The temporal schedule of oviposition of first and subsequent clutches differs between first-year females and females that are 2 years old or older (2Y+) (Baird et al., 2001; Telemeco & Baird, 2011). First clutches produced by 2Y+ females typically begin to develop in early May and are oviposited about 2 weeks later during the third week of May. First-year females ripen their first clutches 10–15 days later in mid-May, and lay them at the end of May or the first week of June. Females of both age groups may produce second or third clutches (Baird, 2004; Telemeco & Baird, 2011), which are oviposited throughout June, into the first 2 weeks of July. Once the last clutches of the season are oviposited, females feed heavily and become progressively less active to store energy for winter (Baird & Sloan, 2003).

Eggs from the earliest clutches begin to hatch in mid-July, and hatchlings from later clutches continue to emerge until mid-October. Offspring are 38.0–40.0 mm in snout–vent length (SVL) when they hatch, and may grow to 70.0–85.0 mm SVL by the end of the active season in late October. From 15 July–15 October we surveyed the study site at least three times per week to capture, mark and measure newly emerged hatchlings. We recorded standard measurements (SVL, tail length, body mass) at first and subsequent captures, determined sex by examination of the postanal scales (enlarged in males), mapped the location of the first and subsequent captures on scale-drawn maps, and collected a blood sample (see Collection of DNA Samples and Determination of Genotypes below). Hatchlings were then released unharmed by placing them in rock crevices at their precise capture locations (see Ethical Note below).

Genetic assignment of hatchlings to individual mothers (see Parentage Assignments and Patterns of Reproductive Success in Females and Males below) of known age, combined with the schedule of oviposition for serial clutches and hatching SVL at first capture, allowed us to estimate whether offspring were from first or subsequent (second or third) clutches. Assignment of offspring to first clutches was obvious using these criteria for both 2Y+ and first-year females. Because third clutches sometimes begin maturation before oviposition of second clutches, and third clutches develop rapidly (Baird, 2004), we could not distinguish hatchlings from second or third clutches. Therefore, we pooled hatchlings from second and third clutches for analyses.

**Collection of DNA Samples and Determination of Genotypes**

We collected a blood sample for isolation of nuclear DNA from all adult lizards (N = 55) when they emerged from hibernacula in early April 2007 and from 86 hatchlings from August 2007 to May 2008 (for details see Results, Schedule of Clutch Production and Hatching Emergence). Blood samples (50 μl) were collected from the orbital sinus using heparinized microhaematocrit tubes, and immediately transferred into 15 ml tubes containing standard lysis buffer for storage until DNA extraction. Bleeding was staunched by applying slight pressure to the orbit with a cloth. We returned lizards to their exact capture locations within 15 min (see Ethical Note below).

Genomic DNA was isolated from blood samples using a DNeasy blood and tissue extraction kit (Qiagen, Venlo, The Netherlands). Using PCR, we amplified 11 microsatellite loci with primers developed for C. collaris (Hutchinson, Strasberg, Brission, & Cummings, 2004) that were labelled with fluorescent dyes. PCR amplification reactions (15 μl) contained 4.75 μl of genomic DNA, 0.50 μl of forward and reverse primers, 9 μl of True Allele Premix (Perkin-Elmer Applied Biosystems, Foster City, CA, U.S.A.) and 0.25 μl of GoTaq DNA polymerase (Promega, Madison, WI, U.S.A.). All PCR products were optimized according to the thermal profile and annealing temperatures described in Husak, Fox, Lovern, and Van Den Bussche (2006). We visualized amplicons for all loci on an automated DNA sequencer (model ABI 3130, Perkin-Elmer Applied Biosystems) after loading a mixture of 9.25 μl of
formamide, 0.25 μl of ROX 500 HD size standard and 0.50 μl of PCR product. Each mixture was denatured at 96 °C for 5 min and then immediately chilled on ice for 3 min prior to loading. We scored all genotypes using GeneMapper software version 4.0 (Perkin-Elmer Applied Biosystems). To test for the presence of null alleles, large-allele dropout, and stutter-induced typing errors at each locus, we used Microchecker software version 2.2.1 (University of Hull, Yorkshire, U.K.; Van Oosterhout, Hutchinson, Willis, & Shipley, 2004). Therefore, we used the locations of adults relative to hatch-parents, all hatchlings that were homozygotes at these two loci within 20 m of their first capture location for 1–1.5 months (Baird, n.d.). Therefore, we used the locations of adults relative to hatchlings as the first criterion to inform our analysis of possible parents (also see Zamudio & Sinervo, 2000). Our two smallest habitat patches (1230 and 1505 m²) were separated by only 40 m of grass. All adult males (N = 27) and hatchlings (N = 86) in the 2007 cohort were genotyped for all 11 loci. We genotyped all 28 females that were present in 2007, but only included in our parentage analyses the 18 that produced clutches (see Results, Schedule of Clutch Production and Hatchling Emergence). Two loci (Orig11, ENR21) showed a high frequency of null alleles. Null alleles can confound parentage assignments when a true heterozygote is incorrectly typed as a homozygote, potentially resulting in a genetic mismatch and false exclusion of the true parent (Dakin & Avice, 2004). However, because both loci were polymorphic, we retained them in analyses. To avoid false exclusion of potential parents, all hatchlings that were homozygotes at these two loci (N = 66) were typed at only one allele for parentage analyses following the methods of LeBas (2001) and Husak et al. (2006).

Parentage Assignments and Patterns of Reproductive Success in Females and Males

Both adult female and male collared lizards maintain strong philopatry to home ranges and territories, and mark–recapture/mapping studies since 1990 have shown that hatchlings remain within 20 m of their first capture location for 1–1.5 months (Baird, n.d.). Therefore, we used the locations of adults relative to hatchlings as the first criterion to inform our analysis of possible parents (also see Zamudio & Sinervo, 2000). Our two smallest habitat patches (1230 and 1505 m²) were separated by only 40 m of grass, with a concrete wall running between them, along which we had observed lizards travelling two to four times per season (Curtis & Baird, 2008). We pooled lizards from these two patches for parentage analyses because the potential for gene flow is high. There was also a much larger (19 583 m²) habitat patch, 260 m from the other two at the closest point (Baird & Curtis, 2010), and no more than one lizard per season moved between this larger patch and the other two during 17 seasons, and no such movements were recorded in 2007. Consequently, we considered the larger patch separate from the small patch for parentage analyses.

For these two pools of potential parents and offspring, we used the maximum likelihood program CERVUS version 3.0 (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton, 1998) to assign a mother and father for all hatchlings at 80% confidence. Our simulation parameters for parentage assignments were as follows: (1) mean proportion of candidate fathers (or mothers) sampled = 0.98, (2) proportion of loci mistyped = 0.01 and (3) number of simulation cycles = 100 000. For hatchlings that CERVUS could not assign to a mother and/or father at 80% confidence, we compared the genotypes of the two most likely parents to the hatchling genotype, and excluded the parent candidate that mismatched at any locus (genetic exclusion; Haynie, Van Den Busse, Hoogland, & Gilbert, 2003). If the two most likely parents did not mismatch, or shared the same number of mismatches with that of the hatchling at any locus, we measured the minimum linear distance between the mapped first hatchling capture location and the nearest boundaries of the home ranges (or territories) of each candidate parent, and assigned the hatchling to the parent that was closest.

We estimated relative fitness for individual males for all clutches pooled, and for the first and subsequent (second and third) clutches separately by dividing absolute reproductive success (number of offspring sired) by the population mean reproductive success for males. We estimated the number of different males that females mated with by determining the identities of sires for the offspring assigned to individual females, and the number of different males that inseminated individual clutches by determining the identity of sires for hatchlings from individual clutches.

Statistical Analysis

All analyses were performed in the program R version 3.0.1 (R Development Core Team, 2013). We used generalized linear models (GLM) to compare the following variables in 2Y-+ males that were defending territories and first-year males that were not: hourly rate of patrol (m/h), hourly frequencies of broadcast display, courtship encounters with females, contests with rival males, territory/home range area (m²), the number of females that males overlapped spatially and SVL (mm). Because the number of females overlapped was a discrete variable, we used a GLM with Poisson error structure and log link function. The response variables for all other GLMs were continuous, so we used a Gaussian error structure and identity link function. We also used GLM to compare relative fitness in territorial and nonterritorial males by including sire social status (territorial or nonterritorial) as a categorical predictor and relative fitness as a response variable with Gaussian error structure and identity link function. We used the package compute.es to calculate standardized (mean = 0, standard deviation = 1) effect sizes (Cohen's d) and associated 95% confidence intervals (CI) for all models.

To determine the strength of selection acting on phenotypic traits that are hypothesized to promote fitness in male lizards (reviewed by Baird, 2013c), we calculated standardized linear selection differentials (ŝ) using the methods of Morrissey and Sakrejda (2013). This approach employs spline-based generalized additive models to model absolute fitness as a function of unstandardized traits, and converts regression coefficients of unstandardized traits into standardized linear selection differentials. To avoid problems with model overfitting and multicolinearity among independent variables, we conducted separate univariate analyses of selection for individual traits (see Bolund, Bouwhuys, Pettay, & Lummaa, 2013). We used the package mgcv to model absolute reproductive success as a function of male phenotypic traits. Standardized linear selection differentials and standard errors were estimated from models after 1000 parametric bootstrap replicates in the package gsg (Morrissey & Sakrejda, 2013).

For our selection analyses, we chose traits that are hypothesized to be important components of male fitness in our study population (Baird et al., 2003; Baird et al., 2007) and lizards in general (Andersson, 1994; Zamudio & Sinervo, 2003; reviewed by Baird, 2013c). Baird et al. (2007) showed that broadcast display, patrol and courtship behaviours are positively associated with annual male mating success as estimated by relative courtship frequency in the Arcadia Lake population, whereas intrasexual contests and large body size (SVL) do not influence mating success. Especially in territorial polygynous species such as collared lizards, the number of females overlapped has been used as an estimate of male fitness because males overlapping more females are assumed to mate more frequently and hence achieve higher reproductive success (Andersson, 1994; Lappin & Husak, 2005). Therefore, on the basis of previous behavioural studies we predicted that the frequency of broadcast display, patrol rate, frequency of courtship and the number of females overlapped would be under positive directional selection, whereas traits that previous studies have not linked with
behavioural estimates of mating success (male SVL, frequency of contests initiated) would not.

For all analyses, our primary means for evaluating statistical significance involved determining whether the 95% CI of predictor variables did not include zero. However, because our selection analyses involved multiple univariate tests, we also used Bonferroni correction for \( P \) values as a supplementary measure of statistical significance. For all other analyses, we do not report \( P \) values, because they are subject to sample size variation and do not reveal the biological significance or statistical uncertainty of the variables of interest (Colegrave & Ruxton, 2003; Nakagawa & Cuthill, 2007). Instead, we use standardized effect sizes with CI because they provide both biologically and statistically meaningful inference at a specified degree of certainty (Colegrave & Ruxton, 2003) and have the additional advantage of facilitating direct comparisons of the influence of variables in our system with other systems (Nakagawa & Cuthill, 2007).

Ethical Note

All procedures performed on live lizards were conducted with approval of the Institutional Animal Care and Use Committee of the University of Central Oklahoma (IACUC, permit number 13009) and the Oklahoma Department of Wildlife (permit number 5553). We have conducted 17 consecutive seasons of longitudinal studies of collared lizard behaviour, growth and survival in this population. We clipped the terminal phalanges of three digits for permanent identification of individuals, applied nontoxic acrylic paint to the dorsum for identification of individuals from a distance, repeatedly captured individuals by noosing, palpated female abdomens to monitor reproductive cycles of females, and made morphometric measurements on lizards at each capture. Subsequent monitoring of the behaviour of lizard subjects confirmed that these techniques have no adverse effects on the health of adult or hatchling collared lizards (Baird, 2004; Baird et al., 2001; Baird et al., 2003; Baird et al., 2007). Collection of blood from the orbital sinus using a micro-haematocrit tube is the most humane technique, because samples are taken within 1 min of capture, and bleeding is staunched quickly (\( \leq 1 \) min) by applying gentle pressure to the orbit with the eyelids closed. Alternative collection techniques (e.g. from clipped toes, needle puncture) are more invasive and/or would require much longer handling. Rapid blood collection from the orbital sinus is especially important to protect hatchlings because they are the most susceptible to overheating (high surface area/volume) during handling. We have blood-sampled hundreds of hatchlings from 2007 to 2013 and subsequently monitored their behaviour for the balance of these seasons and beyond with no indication of ill effects. Hatchlings are almost always resighted behaving normally within 1 h of blood sampling, and the others are sighted no more than 2 days later. We have not observed one incidence of eye infection in the hundreds of lizards that we have blood-sampled.

RESULTS

Male Spatial and Social Behaviour

Consistent with findings of past studies on male behaviour at Arcadia Lake, all 2Y+ males (\( N = 17 \)) defended territories, whereas even though they were sexually mature, first-year males (\( N = 10 \)) did not. Average rates of patrol and broadcast display in territorial males were 5.2 times and 3.1 times higher, respectively, (patrol: GLM: \( t_{25} = 7.27; \text{Cohen’s } d = 2.90, 95\% \text{ CI } = 1.74–4.05 \); broadcast display: GLM: \( t_{25} = 6.50; \text{Cohen’s } d = 2.59, 95\% \text{ CI } = 1.49–3.68 \); Table 1). On average, territorial males courted females 3.2 times more frequently (GLM: \( t_{25} = 2.59; \text{Cohen’s } d = 1.03, 95\% \text{ CI } = 0.16–1.90 \)) than did nonterritorial males (Table 1). Not only did territorial males initiate aggressive contests more frequently (GLM: \( t_{25} = 3.71; \text{Cohen’s } d = 1.48, 95\% \text{ CI } = 0.56–2.40 \); Table 1), in most contests (95%) they engaged nonterritorial males that fled immediately. Territorial males were significantly larger (GLM: \( t_{25} = 5.58; \text{Cohen’s } d = 2.33, 95\% \text{ CI } = 1.28–3.38 \) than nonterritorial males (Table 1). In marked contrast, both the size of areas used (GLM: \( t_{25} = 0.34; \text{Cohen’s } d = 0.14, 95\% \text{ CI } = –0.06–0.06 \)) and the number of females overlapped (GLM: \( t_{25} = 0.03; \text{Cohen’s } d = 0.01, 95\% \text{ CI } = –0.01–0.01 \)) were similar in the two social classes of males (Table 1).

Schedule of Clutch Production and Hatching Emergence

Of the 28 females that emerged from hibernacula in early April of 2007, 10 died before they produced a first clutch, as indicated by their abrupt and continued absence. We monitored the reproductive cycles of the remaining 18 females using recaptures from 1 May to 15 July during which we palpated the abdomen to assess developmental stages and oviposition of one to three clutches of eggs. These 18 females produced 25 clutches total. Eight females produced only one clutch because they died before the reproductive season was completed, whereas seven and three females that survived the entire season produced two and three clutches, respectively. The eight females that died early were each captured and palpated three to four times. The remaining 10 females were palpated 8–12 times throughout the season.

Daily monitoring revealed that, in 2007, hatchlings began emerging from the earliest clutches on 13 August. We captured 70 hatchlings from then until October 2007, and we captured an additional 16 hatchlings from 2007 during 19 April–15 May 2008. Hatchlings that we captured first in 2007, as well as those captured in early 2008, were distributed randomly throughout the entire site. Therefore, the fact that we did not capture 19% of hatchlings until 2008 does not introduce systematic bias into our estimates of male reproductive success. Hatchlings from later clutches began to emerge on 10 September, which resulted in their being substantially (<20 mm) smaller than already emergent hatchlings from first clutches. Based on the different reproductive schedules of first- and 2Y+ females (see Methods, Schedule of Egg Production and Hatching), and the marked difference in the size of hatchlings when they first emerged, we were able to assign 68 offspring to first clutches and 18 offspring to subsequent (second or third) clutches.

Parentage Assignments, Mating Relationships and Reproductive Success in Males and Females

We assigned a father and mother for all hatchlings on the small habitat patch using CERVUS. On the large habitat patch, we
assigned 37 (62%) hatchlings a father and 35 (56%) hatchlings a mother using CERVUS. Of the 25 hatchlings that we could not assign a father using CERVUS, eight were assigned using genetic exclusion, whereas 17 were assigned by exclusion based on spatial proximity (see Methods, Parentage Assignments and Patterns of Reproductive Success in Females and Males). Twenty-seven hatchlings could not be assigned a mother using CERVUS. Of these, seven were assigned based upon genetic exclusion and the other 20 were assigned using spatial proximity.

When we pooled data for all clutches, the relative fitness of territorial and nonterritorial males was similar (GLM: $t_{2,25} = -0.17$; Cohen’s $d = -0.07$, 95% CI = $-0.89$–$0.75$; Fig. 1). All nonterritorial males sired at least one offspring, whereas three territorial males did not sire any offspring. Because survivorship of hatchlings from first clutches appears to be higher than that from second and third clutches in the Arcadia Lake population (Baird, n.d.), we examined reproductive success of the two social classes of males from first versus later clutches separately. Relative fitness of males adopting the two social tactics was also similar for the first clutch alone (GLM: $t_{2,25} = -0.34$, Cohen’s $d = -0.13$, 95% CI = $-0.96$–$0.69$; Fig. 1), as well as for later clutches (GLM: $t_{2,25} = 0.27$; Cohen’s $d = 0.11$, 95% CI = $-0.71$–$0.93$; Fig. 1).

Sixteen females (89%) copulated with two to six males, whereas only two females (11%) mated exclusively with one male (Fig. 2). Of the 16 females mating with multiple males, 13 (81%) mothered hatchlings sired by both territorial and nonterritorial males (Fig. 2). Seventy-six per cent (19 of 25) of individual clutches were fertilized by multiple (up to six males) and eggs in 15 (60%) clutches were fertilized by both territorial and nonterritorial males (Fig. 3).

Because the number of offspring sired by territorial and nonterritorial males was not different, we pooled them for our estimates of the strength of sexual selection on male phenotypic traits even though behavioural and morphological traits were markedly different. As a consequence of these differences, none of the seven traits that we examined were significant targets of selection for all males pooled ($P_{s} 0.22$–$0.90$; Table 2). When only territorial males were considered, we found positive directional selection on male SVL ($P = 0.02$; Table 2), but Bonferroni correction rendered this result nonsignificant.

![Figure 1.](image1.png) **Figure 1.** Relative fitness in territorial (solid bars) and nonterritorial (hatched bars) male collard lizards for all clutches pooled, first clutches only and the second and third clutches pooled. Values are means ± SE.

![Figure 2.](image2.png) **Figure 2.** Number of female collard lizards that produced offspring with one male versus multiple males. Hatched bars: females that mated only with territorial males; open bars: females that mated only with nonterritorial males; solid bars: females that mated with territorial and nonterritorial males.

**DISCUSSION**

As expected, 2Y+ males vigorously defended territories and courted females that resided within their territories far more frequently and conspicuously than first-year males that displayed subordinate nonterritorial tactics. Nevertheless, genetic parentage analyses did not support predicted patterns of reproductive success based on these patterns of male behaviour. Territorial males did not effectively monopolize matings with the females that resided within their territories. Instead, females were highly promiscuous, producing offspring with several other males (even for individual clutches), both territorial and nonterritorial. Nonterritorial males obtained unexpectedly high reproductive success, siring equal numbers of offspring on average relative to territory owners. That nonterritorial males had equal reproductive success while avoiding the costs of territory defence incurred by territory owners (Baird et al., 2001), and that females did not maintain high mating fidelity to territory owners, calls into question the adaptive value of territory defence in this population. These results are surprising, because field studies in all populations of *C. collaris* examined to date show that the largest males incur substantial costs by defending reproductive territories, and hence are expected to obtain a disproportionate reproductive advantage over nonterritorial males to balance these costs (Baird et al., 2003; Lappin & Husak, 2005; McCoy, Baird, & Fox, 2003).

One possible explanation of the unexpectedly high reproductive success of nonterritorial males is that the habitat topography at Arcadia Lake promotes exceptional opportunities for these males to travel to contact females and mate by stealth. Habitat patches at Arcadia Lake are large and continuous and contain nearly unlimited crevices in which nonterritorial males can hide to evade attacks by territorial males without being forced to disperse away from females (Baird & Sloan, 2003). Especially when territorial males are patrolling and displaying at distant boundaries, there appear to be opportunities for nonterritorial males to sneak copulations. By contrast, the natural habitat of collared lizards is fundamentally different from that at Arcadia Lake. It consists of smaller
Olsson, 2005), there is little support for the hypothesis that multiple males (Arnqvist & Nilsson, 2000). With a few exceptions (Uller, 2000), multiple males (sperm competition), or bias fertilization by sperm obtained from particular males (cryptic female choice) (Uller & Olsson, 2008). Both of these mechanisms are possibilities in collared lizards. However, differences in relative testis size between males using different social tactics, which is often observed in species with sperm competition (Birkhead & Møller, 1998), was not observed in territorial and nonterritorial males from the Arcadia Lake population (Baird et al., 2003). In many female squamates having cryptic female choice, there are morphological specializations for sperm storage and/or manipulation, but these are also lacking in female collared lizards (Telemeco & Baird, 2011). Moreover, both sperm competition and cryptic choice models predict random mating by females, but nonrandom biases in paternity (Uller & Olsson, 2008). The distribution of paternity observed in our study did not support either prediction.

Females mated with males displaying a wide range of phenotypic traits. When all males were pooled, none of the traits that are typically hypothesized to promote fitness in male lizards (Baird et al., 2003; Baird et al., 2007; Zamudio & Sinervo, 2003; reviewed by Baird, 2013c) were under strong sexual selection. However, when we restricted the analysis to only males that controlled territories, we found positive directional selection on sire SVL. Large body size among territory owners may promote success in intrasexual contests among males. In many lizard species, larger males often defend larger or higher-quality territories and/or monopolize mating opportunities with more females (Andersson, 1994; Sinervo & Lively, 1996). Sexual selection on body size may be particularly important in species such as collared lizards where males adopt alternative tactics, because traits characterizing each tactic may be under correlational selection. Such a case is evident in water skinks, in which male SVL interacts with a suite of behavioural traits that promote the evolution of divergent social tactics through disruptive selection (Noble, Wechmann, Keogh, & Whiting, 2013).

Sexual selection for large body size among territorial males may also be explained by female choice. If females derive fitness benefits by mating with multiple males, then benefits derived by mating with territorial versus nonterritorial sires are likely different. Females may use body size as an indicator of superior genetic quality in the case of territorial sires (Andersson & Simmons, 2006; Kirkpatrick, 1982) but choose one or more different traits displayed by nonterritorial males. Mating with nonterritorial males also may be advantageous because it promotes genetic compatibility or diversity, both of which may enhance offspring survivorship (Mays & Hill, 2004; Stockley, Searle, Macdonald, & Jones, 1993).

### Table 2

<table>
<thead>
<tr>
<th>Trait</th>
<th>All males pooled (N=27)</th>
<th>Territorial males only (N=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s' (SE)</td>
<td>s' (SE)</td>
</tr>
<tr>
<td>Rate of patrol (m/h)</td>
<td>0.11 (0.17)</td>
<td>0.11 (0.42)</td>
</tr>
<tr>
<td>Broadcast displays/h</td>
<td>0.09 (0.16)</td>
<td>0.27 (0.24)</td>
</tr>
<tr>
<td>Courtship encounters/h</td>
<td>0.22 (0.15)</td>
<td>0.42 (0.23)</td>
</tr>
<tr>
<td>Contests/h</td>
<td>0.18 (0.15)</td>
<td>0.15 (0.23)</td>
</tr>
<tr>
<td>Number of females overlapped</td>
<td>0.07 (0.18)</td>
<td>0.33 (0.35)</td>
</tr>
<tr>
<td>SVL (mm)</td>
<td>0.65 (0.40)</td>
<td>0.60 (0.23)</td>
</tr>
</tbody>
</table>

SVL: snout–vent length.
- Statistically significant difference (95% CI did not include zero).

![Figure 3](image_url)  
**Figure 3.** Number of individual collard lizard clutches inseminated by one male versus multiple males. Hatched bars: clutches inseminated only by territorial males; open bars: clutches inseminated only by nonterritorial males; solid bars: clutches inseminated by territorial and nonterritorial males.
References


Baird, T.A. (n.d.). Determination of dispersal from natal sites and influence of hatching date on offspring survival in hatchling collared lizards described in the present study. Unpublished raw data.


