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Differential sex allocation in sand lizards: bright males induce daughter production in a species with heteromorphic sex chromosomes

Mats Olsson[1,2,*], Erik Wapstra[3] and Tobias Uller[1,2]

[1]*Department of Zoology, University of Gothenburg, 413 90 Gothenburg, Sweden*
[2]*School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia*
[3]*School of Zoology, University of Tasmania, Hobart, TAS 7001, Australia*
*Author and address for correspondence: School of Zoology, University of Tasmania, Hobart, TAS 7001, Australia (molsson@uow.edu.au)

In sand lizards (*Lacerta agilis*), males with more and brighter nuptial coloration also have more DNA fragments visualized in restriction fragment length polymorphism analysis of their major histocompatibility complex class I loci (and, hence, are probably more heterozygous at these loci). Such males produce more viable offspring, with a particularly strong viability effect on daughters. This suggests that females should adjust both their reproductive investment and offspring sex ratio in relation to male coloration (i.e. differential allocation). Our results show that experimental manipulation of partner coloration in the wild results in significantly higher maternal effort and a 10% higher proportion of daughters than sons. This supports the hypothesis that females increase their maternal energetic expenditure and adjust their offspring sex ratio in response to high-quality partners. However, it also suggests that this has probably evolved through natural selection for increased offspring viability (primarily through production of daughters), rather than through increased mate attraction (e.g. sexy sons).

Keywords: sex allocation; maternal allocation; male attractiveness; major histocompatibility complex; heteromorphic sex chromosomes

1. INTRODUCTION

The differential allocation hypothesis was formulated to explain sex allocation scenarios where partner attraction selects for overproduction of the attractive sex (Burley 1981, 1982, 1986). Since Nancy Burley's first experiments demonstrating greater attraction of male zebra finches (*Taeniopygia guttata*) with orange leg bands, and the corresponding overproduction of sons by females mated to such partners, subsequent studies have given mixed results (Hardy 2002). An elegant study of blue tits in a natural population showed that males deprived of the UV-component in their head colour were less attractive to females and that males with intact UV signals initiated overproduction of sons (Sheldon *et al.* 1999). Similar results were obtained through manipulations of male nuptial coloration in blue tits (*Parus caerulea*; Ellegren *et al.* 1996). However, in barn swallows (*Hirundo rustica*; Saino *et al.* 1999) and dark-eyed juncos (*Junco hyemalis*; Grindstaff *et al.* 2001), no such effect could be established. Thus, although overproduction of sons in response to high-quality partners is the overriding pattern across species in birds (West & Sheldon 2002), why is there substantial variation in research outcome?

As captured by Shaw & Mohler (1953) in their evolutionarily stable strategy analysis of a female's genetic return on investments into grandsons versus granddaughters, her fitness benefit of sex allocation tactics is the summed effect of (facultative or obligate) adjustment at the level of the individual (e.g. with higher returns from more competitive, better-provisioned sons or from more sons than daughters when partners are attractive, i.e. 'sexy sons') and at the level of the population (e.g. when the sex ratio is biased to mismatch population operational sex ratio; e.g. Charnov 1982; Hardy 2002). Therefore, only when the net benefit from sexy sons outweighs other forces of selection will partner attractiveness be identified as a significant predictor of sex ratio adjustment (or statistically teased out as an independent effect). Thus, theory predicts exactly this heterogeneity of sex allocation among taxa and populations—a homogeneous pool of published results would have been more surprising. Furthermore, if one sex were particularly sensitive to maternal effects or genetic viability enhancement from a particularly good or compatible partner genome, then females would be predicted to overproduce that sex. In sand lizards (*Lacerta agilis*), as in birds, females are the heteromorphic sex and, hence, sex-linked genetic pathology probably occurs more in females than in males (Olsson *et al.* 2004, 2005). This is based on the fact that deleterious recessives on the hemizygous part of the Z chromosome are free to express their direct or epistatic negative effects (Olsson *et al.* 2005). Furthermore, female sand lizards that mate with more major histocompatibility complex (MHC) diverse males overproduce daughters, and more MHC diverse males have more nuptial colouration (Olsson *et al.* 2005). The latter of these results seems to suggest that females may not only respond to male genetic cues via odours (Olsson *et al.* 2003), but may also use visual cues for mate quality assessment. We therefore conducted a field experiment in which we first experimentally verified that colour-enhanced males had an increased (for small males quadrupled) reproductive success (see Anderholm *et al.* 2004). Here, we report on how females respond to colour-enhanced males in terms of maternal effort and sex allocation.

2. MATERIAL AND METHODS

The Swedish sand lizard (*L. agilis*) is a sexually dichromatic, up to 20 g, 90 mm snout to vent, ground-dwelling lizard species. Experimental techniques and protocols have been described in detail

elsewhere (Anderholm *et al*. 2004). We therefore only present a summary here.

Sand lizards were captured by noosing or hand at the Asketunnan study site, 50 km south of Gothenburg on the Swedish west coast in the first week of May 2002. A number of morphological measurements were made: snout–vent length to the nearest mm, mass to the nearest 0.01 g (Mettler electronic balance, and head width and length to the nearest 0.01 mm (using digital callipers). The lizards were marked dorsally with an oval piece of fabric tape (TESA tape, Germany; *ca* 10×20 mm) painted with an individual number.

Males were then randomly assigned to either of the treatment categories 'badge-enlarged' versus 'controls', with badge-enlarged males being painted on the body sides with biologically inert, hence harmless, green human tattoo colour. Three different tattoo colours were mixed to match (by eye) a reflectance spectrogram of an adult male sand lizard in the upper quartile of the size and age distribution (Spuck Baulding, New York, colour nos 9046 'emperor green', 9029 'parrot green' and 9028 'radiant green'). Every second male (control) was painted with water and dried. Following painting, all males were sprayed on their body sides with moisture vapour permeable spray dressing (Smith & Nephew, Hull, England). This fixes the tattoo paint and increases its durability in the wild, while forming a corresponding semipermeable skin dressing on control males. Our unpublished spectrophotometry data show that males lack reflectance in the UV spectrum (M. Olsson and S. A. Andersson, unpublished data). We also confirmed that our paint did not reflect in the UV to verify that unidentified UV signalling does not risk confounding our conclusions. Lizards were then released at their site of capture and monitored throughout the mating season.

After copulation, a male usually guards the female for several hours up to more than 10 days (Olsson *et al*. 1996). Considering that copulation only takes 2–4 min and often goes undetected in the wild, mate guarding allowed us to monitor partner identities. Furthermore, mate guarding has been confirmed with molecular genetics techniques (micro- and minisatellites) to accurately reflect paternity (Gullberg *et al*. 1997). Females were captured and marked in the way described for males and were monitored with respect to visiting males throughout the mating season. At the end of the mating season, they were brought into captivity and allowed to oviposit (Olsson & Madsen 2001). Subsequently, the eggs were incubated for *ca* 40 days, and the hatchlings were weighed, measured and sexed (using hemipenis eversion; Harlow 1996), the latter having almost 100% accuracy and repeatability in this species (Olsson *et al*. 2004, 2005).

Treatment effects of badge-enlargement were assessed for sex ratio and maternal energetic expenditure, with females being courted by badge-enlarged males in one category (hereafter 'badge-enlarged'; $n=43$), and females only exposed to control males (heretoforth 'controls'; $n=15$). Maternal investment was estimated by taking residual scores from a regression of clutch mass on female mass following oviposition. Female body condition following egg laying was estimated by taking residuals from a mass–snout–vent length regression. Variables used in parametric statistics were tested for normality using Wilks' lambda tests (Proc Univariate, SAS, 1987). Variables that did not meet the requirements of normality were either successfully normalized (*W*: normal, $Pr<W$, larger than 0.05 in all cases) by transformation or else non-parametric tests were used.

3. RESULTS

Females courted by males with enlarged badges invested significantly more into clutch mass than did their control counterparts (mean residual scores = -0.02 ± 0.16, s.e., versus -0.90 ± 0.53, respectively: $t=2.12$, $p=0.039$). However, because females were also exposed to different numbers of partners and given that we know from previous work that females with more partners produce more viable offspring (reviewed in Olsson & Madsen 2001), we reran this analysis with number of individual partners, and total number of observations with males, in the model. These estimates are not synonymous if females have been observed in multiple copulations or mate guarding with the same male. This analysis confirmed that

females with painted partners were still investing more into egg production than females with control partners ($F_{\text{paint category}}=5.52$, d.f.$=3$, 54, $p=0.022$). Both covariates were non-significant at $\alpha=0.05$ level (number of individual partners $p=0.07$, number of total observations with males $p=0.13$).

To verify that females committed to a more costly strategy when being courted by painted partners, we looked for effects of maternal investment on body condition following oviposition. The direct effect of partner paint category was not statistically significant when number of days kept in captivity (with potential for feeding bias) were included in the model ($F=0.75$, $p=0.39$). Maternal energetic expenditure, however, strongly influenced body condition following oviposition ($F=28.32$, $p=0.0001$). Thus, females investing relatively more into egg production became more energetically depleted.

In a corresponding analysis, we looked for effects of paint category on offspring sex ratio. A first *t*-test demonstrated that females with painted partners produced 10% more daughters than did females with control partners (mean number of young per female = 8.05 ± 0.67, s.e., mean sex ratio = 0.38 ± 0.034, s.e., versus, 0.48 ± 0.036, s.e., respectively, $t=2.04$, $p=0.048$). However, because sex ratio covaried with clutch size (Spearman's correlation analysis, $r_s=0.39$, $p=0.003$, $n=57$) and has been repeatedly shown in other taxa to covary with laying date (Hardy 2002), we reran this analysis with clutch size and laying date entered as covariates. Our analysis held up also in the presence of these potentially confounding factors ($F=6.77$, $p=0.012$; covariate laying date $F=0.30$, $p=0.58$, and clutch size $F=9.51$, $p=0.003$).

4. DISCUSSION

Our previous work shows that female sand lizards respond to male MHC genotype by adjusting the sex ratio of their clutch to produce more daughters when males are more MHC diverse (Olsson *et al*. 2005). Such males also exhibit unusually colourful badges (nuptial coloration), suggesting that this could be another cue to male genetic quality (or *vice versa*). The current study verifies that this could indeed be the case. Females courted by males with experimentally enlarged badges both invested more into reproduction (i.e. depleting more of their energetic resources) and overproduced daughters (i.e. the sex most susceptible to genetic disease).

If probability of paternity is represented by a heavily skewed probability distribution, then some males in the population produce many more offspring than the average male. This is supported in our sand lizard population by a descriptive analysis of the moment statistics of the reproductive success distributions of the different sexes. In females, *g*1 (the third central moment statistic, skewness) equals 0.31, whereas it is 1.53 for males (in a non-skewed distribution $g1=0$). Thus, superficially, males should only be produced either when females can provide maternal resources, or when they have access to a partner of sufficiently high genetic quality for the production of successful sons. However, it is probable that male secondary sex

traits are condition dependent (Andersson 1994) and encoded by multiple genes that also determine viability (or pathology) in daughters. Females could therefore gain more from overproduction of daughters when mating with high-quality partners, representing males with particularly 'good genotypes'; all this takes is a higher net return on investments into daughters (through enhanced viability) than through sons (through partner attraction). Our work (Olsson *et al.* 2004, 2005, in press) has shown that indeed there is reason to believe that daughters are more susceptible to parental genetic quality effects than are sons, which supports differential allocation to daughters when mating with above-average partners.

Our results may also have some bearings on theories of antagonistic sexual selection; that is, where genetic effects are sex specific and result in opposing fitness effects in sons ($+$) and daughters ($-$) when sires have particularly high reproductive success (Fedorka & Mousseau 2004). This theory specifically predicts that daughters from more successful sires should show negative correlations between fitness-linked traits and paternal fitness attributes. Our preliminary data suggest the opposite (Olsson *et al.* 2004, 2005). Work is currently in progress to test this hypothesis using a large dataset of genotyped sand lizards in a natural population.

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