

## MATE CHOICE, GENETIC INCOMPATIBILITY, AND OUTBREEDING IN THE ORNATE DRAGON LIZARD, *CTENOPHORUS ORNATUS*

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**Abstract.**—There has been recent interest in the role genetic incompatibility may play in mate or sperm choice. One source of incompatibility may be too similar or disparate genomes. An isolated population of the ornate dragon lizard, *Ctenophorus ornatus*, was followed over a breeding season and parentage assigned to the offspring using microsatellites. It was found that the adults in the population had an eight per cent chance of mating with a relative. I examined whether *C. ornatus* mate (or fertilize their eggs) with respect to genetic similarity. There was no difference in a female's relatedness to the male in whose territory she resided and her average relatedness to all other males. Neither was there a difference in the relatedness of the male that sired a female's offspring and the female's average relatedness to all other males. There was no evidence of a cost to mating with a more genetically similar mate, because offspring survival was not influenced by degree of inbreeding or outbreeding. However, females that were more outbred had fewer offspring survive. In this small population there are two possible explanations for the decreased fitness associated with outbreeding. First, the species may have an evolutionary history of inbreeding and thus may be susceptible to outbreeding depression. Second, as fitter individuals produce more offspring, these offspring have an increased probability of breeding with relatives, leading to an indirect relationship between fitness and outbreeding.

**Key words.**—Agamidae, genetic incompatibility, genetic similarity, inbreeding, mate choice, outbreeding depression, sexual selection.

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The genetic similarity of parents can have important implications for offspring fitness (Falconer and Mackay 1996; Pusey and Wolf 1996; Ober et al. 1998). A pair's genetic similarity can range from that of close relatives to different species in hybrid zones. In animals, evidence of the fitness consequences of parent relatedness comes mostly from either end of this continuum. Typically, mating between close relatives leads to inbreeding depression, in both domestic animals (Falconer and Mackay 1996) and in natural populations (Jimenez et al. 1994; Keller et al. 1994; Madsen et al. 1996; Saccheri et al. 1998; but see van Noordwijk and Scharloo 1981; Gibbs and Grant 1989). At the other end of the continuum, mating between distantly related individuals can lead to either heterosis or outbreeding depression (Templeton 1986). Between these extremes a pair can have a range of relatedness values but the effects of these intermediate levels of genetic similarity on fitness are not so well documented (but see Bensch et al. 1994). Nevertheless, it has been suggested that animals may seek an "optimal level of outbreeding" with the idea that there is a middle point of relatedness that maximizes fitness (Shields 1982; Bateson 1983).

Evidence that animals prefer to mate with intermediate relatives has been found in laboratory choice experiments in a range of species (Bateson 1982; Barnard and Fitzsimons 1988; Burley et al. 1990; Keane 1990). The results of these studies suggest that there may be a disadvantage to mating with individuals too distantly related. However, although optimal outbreeding and outbreeding depression is well established in plants (Waser 1993), in animals there appears to be little evidence of outbreeding depression on a local scale (Pusey and Wolf 1996, but see Brown 1991; Knowlton and Jackson 1993). This lack of evidence has led to suggestions

that preference for immediate relatives may be more a consequence of adaptation to ensure adequate (i.e., no matings with close kin or different species) rather than optimal outbreeding (Pusey and Wolf 1996).

There has been recent interest in the genetic incompatibility of a mating pair, in which one potential source of the incompatibility is too similar or too disparate genomes (Zeh and Zeh 1996, 1997). In a number of studies females mated to multiple males had higher offspring viability (review Tregenza and Wedell 2000). Increased viability does not appear to be a consequence of material benefits or the result of males with good genes conferring viability advantages to all females. These studies have been interpreted as evidence of a role for genetic incompatibility in mating outcomes. Although genetic incompatibility may operate through a range of mechanisms, the genetic similarity or relatedness of parents is a likely source of incompatibility (Tregenza and Wedell 2000). Indeed, some of the strongest evidence for genetic incompatibility comes from an inbred population of sand lizards, *Lacerta agilis*, in which females show sperm choice for less genetically similar males (Olsson et al. 1996a,b).

Inbreeding in populations has usually been studied by long-term detailed pedigree studies using inbreeding coefficients (van Noordwijk and Scharloo 1981; Gibbs and Grant 1989). With the advent of molecular techniques, individual heterozygosity (the proportion of heterozygotes to homozygotes) has provided a measure of recent inbreeding without the need for detailed pedigree studies. A new measure specifically for microsatellite data, mean  $d^2$ , is a measure of the genetic distance between parental gametes and is likely to reflect the extent of population mixing (Pemberton et al. 1999). Mean  $d^2$  is based on the stepwise model of mutation for microsatellites and is the square of the distance in repeat units between alleles averaged over all loci (Coulson et al. 1998). Studies thus far on red deer, *Cervus elaphus*, (Coulson et al. 1998, 1999), harbor seals, *Phoca vitulina*, (Coltman et al. 1998) and

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TABLE 1. Characteristics of *Ctenophorus ornatus* microsatellite loci. Repeat motif is for the sequenced allele. The size range, number of alleles, expected ( $H_E$ ), and observed ( $H_O$ ) heterozygosity were estimated from 79 individuals (Co12E10,  $N = 78$ ).

| Locus   | Repeat motif   | Size range (bp) | No. of alleles | $H_O$ | $H_E$ |
|---------|--|-----------------|----------------|-------|-------|
| Co10F11 | (TC) <sub>17</sub>   | 90–122          | 11             | .772  | .743  |
| Co6A6   | (TC) <sub>10</sub> (AC) <sub>9</sub> (AG) <sub>15</sub>                        | 130–168         | 8              | .646  | .674  |
| Co9C11  | (AG) <sub>17</sub>   | 163–181         | 7              | .969  | .747  |
| Co12E10 | (GT) <sub>13</sub>   | 149–209         | 12             | .679  | .818  |
| Co7G1   | (TC) <sub>4</sub> (GT)(TC) <sub>8</sub>  | 187–203         | 5              | .462  | .396  |
| Co12H6  | (AC) <sub>22</sub>   | 167–205         | 12             | .842  | .909  |
| Co11C3  | (CA) <sub>21</sub> (GA) <sub>24</sub> (GT)(AC) <sub>15</sub> (AG) <sub>4</sub> | 159–209         | 14             | .873  | .842  |

Arabian oryx (Marshall and Spalton 2000) have found relationships between mean  $d^2$  and fitness, suggesting that mean  $d^2$  incorporates information on genetic makeup that has important fitness consequences for individuals.

In this study I examine the relationships between genetic similarity, mate choice and fitness in a natural population of the agamid lizard, *Ctenophorus ornatus*. *C. ornatus* is a territorial species that inhabits isolated granite outcrops in southern Western Australia. Males maintain territories in which females reside. I examined the relatedness of the adults in the population, whether genetic similarity influenced mate choice and the fitness consequences of inbreeding and outbreeding. I investigated two ways in which lizards could avoid mating with genetically incompatible individuals. First, kin may be recognized and avoided during territory establishment. Second, lizards may reside in relative's territories but choose to mate with unrelated individuals or mate with a range of males (polyandry) and exercise female choice though genetic compatibility (Olsson et al. 1996b; Zeh and Zeh 1996, 1997). I examine the fitness consequences of inbreeding and outbreeding by examining the relationships between both offspring and adult fitness and their genotype.

## MATERIALS AND METHODS

### Field Site and Methodology

This study was conducted at Tutanning Nature Reserve 200 km southeast 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 m<sup>2</sup> granite outcrop were marked with two different color 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 emergence. Immediate processing prior to the time of first lizard activity ensured the study process did not influence male territory maintenance. The continuous collection regime resulted in recapturing most animals approximately every two weeks, which was necessary to monitor female reproductive condition and approaching sexual maturity of juveniles.

A grid that encompassed the entire outcrop was marked at five meter intervals for 145 m east-west and 100 m north-south. The lizards were normally active during the early morning and late afternoon and sought cover from the heat during the middle of the day. I observed the lizards during

periods of peak activity, typically three hours in the morning and three hours in the late afternoon. During observations I recorded the lizards' locations on the grid for determination of territory size.

To obtain a true, undisturbed measure of reproductive success females were allowed to lay naturally in the field. This methodology ensured there were no territory disturbances or consequent artificial increases in lizard interactions due to female lizards being removed from the outcrop to lay. Females become dusty when digging egg chambers and I recorded when a female first appeared dusty as her laying date. Because the outcrop was observed continuously (typically 12 days of observations followed by two days away, repeated over six months), offspring could be collected as soon as they hatched. That this method was successful at collecting offspring immediately following hatching was evidenced by the umbilical scar not being closed over in the majority of offspring (39 of 50) at the time of collection. Offspring in which the umbilical scar was closed over were excluded from all analyses that required a morphological measurement at hatching.

### Microsatellites and Parentage Assignment

Seven microsatellite markers that had been developed for *C. ornatus* (Table 1; LeBas and Spencer 2000) were used to assign parentage to offspring. Microsatellite markers were run according to the protocol of LeBas and Spencer (2000) and parentage assigned using CERVUS (Marshall et al. 1998) as described in LeBas (2001). One locus (pCo12E10) had a high null allele frequency (LeBas 2001). Adults that were known to have a null allele from parentage assignments were recorded as heterozygous with allele 'n' (which is treated like any other allele) for relatedness analyses.

### Relatedness and Dispersal

Relatedness between lizards was examined using the programs Relatedness 5.0.4 (Queller and Goodnight 1989) and Kinship 1.2 (Goodnight and Queller 1999). I used the simulate function of Kinship 1.2 to determine the 95% confidence limits of unrelated ( $r = 0$ ) pairs and classified any pairs with a higher relatedness value than the positive confidence limit as related. A lizard's chance of mating with kin was calculated as the number of male-female pairs significantly related, divided by all possible opposite sex pairs.

To determine if males and females differed in dispersal, the average relatedness within each sex was compared to that between the sexes. Standard errors for average relatedness

values were obtained by jackknifing over loci. It was assumed that the jackknife pseudovalues followed a  $t$  distribution, with one degree of freedom less than the number of loci jackknifed over (Queller and Goodnight 1989). Average relatedness in the different groups was compared with an unpaired  $t$ -test using the jackknifed standard errors. Due to unequal variances between the male-male and male-female relatedness distributions ( $F[6,6] = 5.15$ ,  $P = 0.03$ ) a Welch's approximate  $t$  was performed for this comparison (Zar 1984). This difference in variance is likely to be a side effect of the relatedness calculations. Male-female relatedness can be determined either asymmetrically (male relatedness to females only or female relatedness to males only) or symmetrically (both directions) (Relatedness 5.0, Queller and Goodnight 1989). For *C. ornatus* it is the symmetric calculations that generate a small standard error; neither of the asymmetrical standard errors differ from those of the within sex (female-male versus within males  $F[6,6] = 2.31$ ,  $P = 0.17$ ; male-female versus within males  $F[6,6] = 1.33$ ,  $P = 0.37$ ). As all three comparisons are valid for determining relatedness, but the symmetrical relatedness uses the available information more thoroughly (Relatedness 5.0, Queller and Goodnight 1989), an unequal variances test was employed, but the unequal variances are best not considered as biologically meaningful.

#### Mating Patterns

Pairwise relatedness values were used to compare the relatedness of females to their territorial males, offspring sires, and average relatedness to all other males in the population. As females lay up to three clutches a season, each clutch was considered separately unless it involved the same pair. A female's territorial male was the male whose territory had the greatest overlap with the female's territory. For details of territory analysis see LeBas (2001). In cases in which male territory shifts over time meant that two males had a greater than 50% overlap of the female's territory, the female's relatedness to these males was averaged. In clutches with multiple paternity the female's relatedness to the sires was averaged.

#### Measures of Inbreeding and Outbreeding

Two measures of an individual's genotype were calculated: heterozygosity and mean  $d^2$ . The first measure, individual heterozygosity, is the proportion of heterozygous loci. The second, mean  $d^2$  is the squared distance between alleles at each locus averaged over all loci for which the individual was typed (Coulson et al. 1998). Due to large variation between loci in  $d$ , which is further amplified by squaring, one locus can heavily weight the measure. Because  $d^2$  is averaged, this can create problems when typing is incomplete. Individuals missing the locus that is heavily weighing the measure can have deflated  $d^2$  measures purely as a result of their incomplete typing. Attempts to standardize  $d^2$  across loci have resulted in the relationships between  $d^2$  and fitness being removed, suggesting that important information is lost in the standardization (Coltman et al. 1998; Coulson et al. 1999). I accounted for these problems by not including loci that were not typed for all individuals and calculated mean  $d^2$  for

the remaining loci. Therefore, mean  $d^2$  for the adults is the mean of six loci (pCo12E10 excluded), and for the offspring, of five loci (pCo12E10 and pCo11C3 excluded).

#### Offspring Survival

I examined whether an offspring's genotype, measured as mean  $d^2$  or heterozygosity, influenced offspring survival. Survival was measured as the number of weeks an offspring lived after hatching, censored at 15 weeks. Data were censored at 15 weeks, because at this age the size of a fast-growing offspring was equivalent to the smallest-sized lizard that arrived at the outcrop during the study. Therefore 15 weeks was considered the age at which offspring had the potential to disperse; consequently, after this time absence from the outcrop could not be attributed to death. Offspring survival was examined with a Cox regression survival analysis (SPSS, SPSS Inc., Chicago, IL.). The survival analysis also incorporated the offspring hatching date and snout vent length, as these variables were likely to influence survival. Snout vent length was measured immediately after hatching for 39 of the 50 offspring (offspring in which the umbilical scar was not closed over at the time of first capture) and hence only these 39 offspring were used in the survival analysis.

#### Adult Fitness

The relationship between the two genetic variables (mean  $d^2$  and heterozygosity) and a measure of adult fitness was examined. The measure of adult fitness was the proportion of an adult's offspring that survived to 15 weeks from their date of hatching (censored at 15 weeks for reasons discussed above).

For analysis using the whole population neither heterozygosity nor mean  $d^2$  were normally distributed. Normality was achieved for mean  $d^2$  by a log transformation (Kolmogorov-Smirnov statistic = 0.083,  $df = 79$ ,  $P = 0.20$ ). Heterozygosity could not be transformed to normality. For regression analyses using only the adults the genetic measures were used untransformed and the residuals of all regressions were normally distributed. As proportions typically have a binomial rather than normal distribution, the proportion of an adult's offspring that survived was arcsine square root transformed (Shapiro-Wilk statistic = 0.882,  $df = 11$ ,  $P = 0.14$ ) (Zar 1984).

## RESULTS

#### Parentage Assignment, Relatedness, and Dispersal

Parentage was assigned successfully to both parents for 44 of the 50 offspring. For details of parentage assignment see LeBas (2001).

For the 29 adults (17 females, 12 males) in the population there were 16 significant pairwise combinations out of a possible 204 that had a relatedness value that fell outside the positive 95% confidence limit for unrelated pairs (positive confidence limit for unrelated [ $r = 0$ ] pairs was 0.386; simulate routine, Kinship 1.2). Therefore, assuming random mating, an individual has a 7.8% chance of mating with a relative in this population.

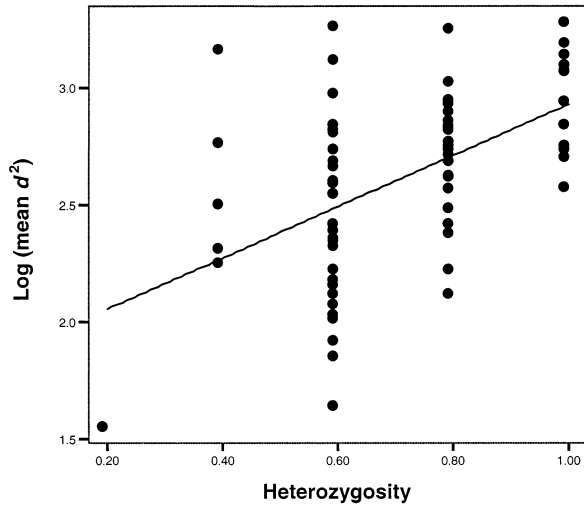


FIG. 1. The relationship between mean  $d^2$  and heterozygosity.

There was no difference in the relatedness within females ( $-0.0164 \pm 0.0292$ ) and the relatedness between males and females ( $-0.0407 \pm 0.0148$ ; unpaired  $t$ -test:  $df = 12$ ,  $t = 0.742$ ,  $P = 0.47$ ). Neither was there a difference in the relatedness within males ( $-0.0749 \pm 0.0336$ ) nor the relatedness between males and females (Welch's approximate  $t$ :  $df = 8.24$ ,  $t = 0.931$ ,  $P = 0.37$ ). There was no difference in the relatedness within females and that within males (unpaired  $t$ -test:  $df = 12$ ,  $t = 1.31$ ,  $P = 0.21$ ). Thus there is no evidence from the genetic data to indicate that males and females differ in dispersal patterns.

#### Genetic Similarity and Mate Choice

There was no significant difference in the relatedness of a female to the male in whose territory she resided (mean =  $-0.0415 \pm 0.0622$ ) and her average relatedness to the other males in the population at the time (mean =  $0.002 \pm 0.0244$ ;  $t$ -test:  $df = 23$ ,  $t = -0.995$ ,  $P = 0.33$ ). Hence territory formation does not appear to be influenced by genetic similarity. Neither was there a difference between the relatedness of a female to the sire of her offspring (mean =  $-0.0533 \pm 0.0496$ ) and her relatedness to her territory holding male (mean =  $-0.0415 \pm 0.0622$ ,  $df = 19$ ,  $t = -0.858$ ,  $P = 0.40$ ) or her relatedness to the rest of the males (mean =  $0.002 \pm 0.0244$ ,  $df = 19$ ,  $t = -1.321$ ,  $P = 0.20$ ). Thus females do not appear to actively seek male sires on the basis of genetic similarity.

#### Genetic Variables and Offspring Survival

Individual heterozygosity was significantly related to mean  $d^2$  in the predicted direction (Fig. 1; Spearman rank correlation:  $N = 79$ ,  $r = 0.50$ ,  $P = 0.001$ ). Survival analysis was used to determine if either of the genetic variables influenced offspring survival. There was no relationship between any of the genetic or morphological variables and offspring survival (Table 2).

TABLE 2. Cox regression analysis of the variables examined for an influence on offspring survival with estimated regression coefficient ( $\beta$ ), standard errors (SE), chi-square statistic (Wald), and significance ( $P$ ).  $N = 39$  hatchlings; for each covariate,  $df = 1$ .

|                   | $\beta$ | SE    | Wald  | $P$   |
|-------------------|---------|-------|-------|-------|
| Date of hatching  | -0.014  | 0.010 | 2.113 | 0.146 |
| Snout vent length | -0.114  | 0.134 | 0.720 | 0.396 |
| Heterozygosity    | -1.484  | 1.433 | 1.073 | 0.300 |
| Mean $d^2$        | 0.793   | 0.718 | 1.220 | 0.269 |

#### Genetic Variables and Adult Fitness

The proportion of a female's offspring that survived to 15 weeks was negatively related to her mean  $d^2$  (Fig. 2), but showed no relationship to her heterozygosity (Table 3). In a multiple regression, female mean  $d^2$  predicted the proportion of her offspring that survived, independent of the mean offspring mean  $d^2$  (whole model:  $F[2,8] = 7.24$ ,  $r^2 = 0.64$ ,  $P = 0.016$ ; female mean  $d^2$ ,  $\beta = -0.0057 \pm 0.0021$ ,  $t = 2.69$ ,  $p = 0.028$ ; mean offspring mean  $d^2$ ,  $\beta = 0.0001 \pm 0.0004$ ,  $t = 0.283$ ,  $P = 0.78$ ). For the males, no genetic variable was related to a male's proportion of offspring surviving (Table 3). Low sample sizes for males temper these conclusions.

#### DISCUSSION

This study documents a risk of inbreeding in a natural population of *C. ornatus*, but finds no influence of genetic similarity on mate choice, measured as either spatial association or offspring parentage. There was no evidence for a cost to mating with a more genetically similar mate because there was no indication of inbreeding depression in this species. In contrast, outbred females had lower reproductive success as measured by the proportion of their offspring that survived.

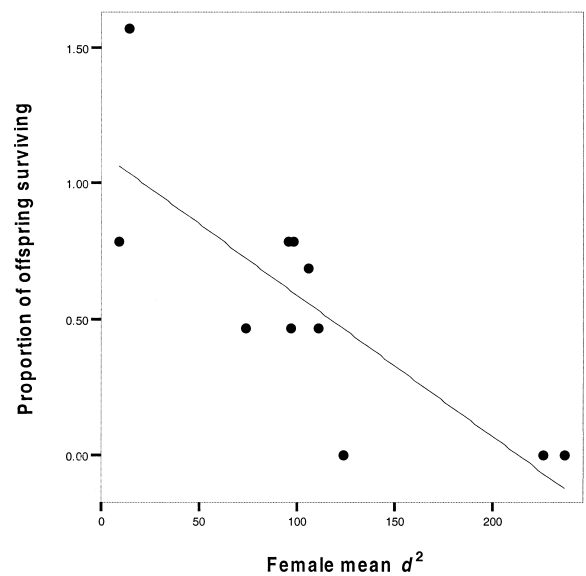


FIG. 2. The relationship between the proportion of a female's offspring that survived to 15 weeks (arcsine square root transformed) and female mean  $d^2$ .

TABLE 3. Least square regressions between the proportion of the adult's offspring surviving to 15 weeks and genetic variables: mean  $d^2$  and heterozygosity. The proportion of offspring that survived is arcsine square root transformed.  $n = 11$  for females and  $n = 7$  for males.

|         |                | $F$   | $\beta + SE$       | $r^2$ | $P$   |
|---------|----------------|-------|--------------------|-------|-------|
| Females | Mean $d^2$     | 16.03 | $-0.005 \pm 0.001$ | 0.63  | 0.003 |
|         | Heterozygosity | 2.71  | $-1.18 \pm 0.716$  | 0.23  | 0.13  |
| Males   | Mean $d^2$     | 0.207 | $0.0005 \pm 0.001$ | 0.04  | 0.67  |
|         | Heterozygosity | 0.000 | $0.006 \pm 0.852$  | 0.00  | 0.99  |

#### Genetic Similarity and Patterns of Mating

In *C. ornatus* there is an approximately eight percent chance of mating with close kin. This constitutes an inbreeding risk, and mate choice for avoiding genetically similar mates in this species may be expected. However, there was no evidence that *C. ornatus* avoid mating with genetically similar individuals when kin are treated as part of a continuum of genetic similarity. Neither did *C. ornatus* avoid mating or siring offspring with more genetically dissimilar individuals. Most studies have not examined mating with respect to genetic similarity as a continuum, but rather as either mating with relatives (of varying degrees) or unrelated individuals (Pusey and Wolf 1996, but see Bensch et al. 1994; Bull and Cooper 1999). However, relatives and unrelated individuals are distributed at each end of a continuum of genetic similarity. Hence, studies examining the genetic compatibility of mates in terms of genetic similarity and those addressing inbreeding/outbreeding avoidance are both addressing the role of genome similarity in mating or fertilization events.

Ralls et al. (1986) proposed that although many species may have a high risk of inbreeding, behavioral avoidance may mean that inbreeding was actually uncommon in natural populations. Studies documenting the observed risk of inbreeding and the actual incidence of inbreeding are rare; however, there is evidence both for (e.g. Høglund 1992; Sillero-Zubiri et al. 1996; Bull and Cooper 1999; Cizek 2000) and against (van Noordwijk and Scharloo 1981; Gibbs and Grant 1989) behavioral inbreeding avoidance in natural populations. *C. ornatus* is territorial and females typically reside within one male's territory (LeBas, unpubl. data). Kin recognition has been documented in reptiles (Bull et al. 1994; Main and Bull 1996) and it was envisaged that *C. ornatus* might avoid residing with close relatives by kin recognition at the time of territory establishment. However, the males whose territories overlapped those of females were no less related to the females than the average male. Differing rates of philopatry between the sexes have also been proposed as a mechanism of inbreeding avoidance. In *C. ornatus* there was no difference in within- and between-sex relatedness, suggesting no difference in dispersal between the sexes in this species.

Females can engage in extrapair copulations to avoid mating with close relatives (Brooker et al. 1990) or may mate multiply to ensure that a genetically compatible sire has been mated (Stockley et al. 1993; Olsson et al. 1996b; Zeh and Zeh 1997). *C. ornatus* females mate with multiple males, as evidenced by 24% of clutches with multiple paternity (LeBas 2001). Additionally, females mate with males other than those that overlap her territory to the extent that only 35%

of clutches are sired solely by a female's territorial male (LeBas 2001). However, these extraterritorial and multiple copulations do not appear to be influenced by genetic similarity. Instead, female behavior may reflect mate choice for secondary sexual traits or male competition for access to females (LeBas 2001).

#### Inbreeding and Fitness

Mating with respect to genetic similarity may be most expected when there is a high risk of mating with a close relative and a cost such as inbreeding depression with such matings. As discussed, there appears to be a risk of inbreeding in this *C. ornatus* population; however, there was no indication of inbreeding depression because heterozygosity was not related to any of the fitness variables. In the sand lizard, *Lacerta agilis*, there are high costs to mating with relatives and females show sperm choice for distantly related males (Olsson et al. 1994; Olsson et al. 1996b). Unlike *C. ornatus*, the sand lizards may be experiencing a recent increase in inbreeding risk due to recent changes in population structure and are possibly currently passing through a bottleneck (Gullberg et al. 1999). Mate choice based on genetic similarity and the consequences of mating with genetically similar individuals are likely to be best understood within the population structure of the species.

#### Outbreeding Depression?

In *C. ornatus* there was no influence of either mean  $d^2$  or heterozygosity on offspring survival. This contrasts with previous studies, which have found positive associations between mean  $d^2$  and fitness; thought to indicate heterosis due to population mixing (Coltman et al. 1998; Coulson et al. 1998). In *C. ornatus*, female fitness, measured as proportion of offspring surviving, is negatively related to mean  $d^2$ . Hence, outbreeding appears to result in a decline in fitness rather than heterosis. That there was a negative rather than a positive effect of outbreeding on offspring fitness in *C. ornatus* is somewhat surprising given the potential for inbreeding in the population.

*C. ornatus* inhabits isolated granite outcrops on which populations as small as the one in this study, a total of 29 breeding adults, are common. Granite outcrops represent islands of suitable habitat, which are often separated by large distances of unsuitable habitat likely to restrict dispersal. Furthermore, land clearing through much of this species' range will have increased the risks of dispersing. Such a population structure implies that *C. ornatus* is likely to have founder effects, continuously pass through bottlenecks, and be subject to genetic drift. That *C. ornatus* persists with such a population structure

suggests this species may have adapted to substantial levels of inbreeding. In support of this notion one of the few bird studies to find no influence of parental genetic similarity on hatching success was on a species subject to population bottlenecks (Gibbs and Grant 1989).

If *C. ornatus* has adapted to high levels of inbreeding, populations may instead be susceptible to outbreeding depression. Low dispersal can cause population subdivision and increase the number of coadapted gene complexes within populations such that there is an increased risk of outbreeding depression (Templeton 1986). The extent to which lizard populations on different outcrops are locally adapted and the degree of gene flow between populations is unknown. Two populations within close spatial proximity differed significantly in the size of sexually selected traits and also parasite load (LeBas, unpubl. data). Such differences on a local scale may influence the success of immigrant individuals that may have smaller sexually selected traits or reduced parasite resistance in the new population. On a wider scale, a study of museum specimens revealed geographic variation in male sexually selected traits such as chest patch area and head depth with both latitude and longitude (Tomkins and LeBas, unpubl. ms.). Previous work (Bradshaw 1963) on *C. ornatus* ecology found that although juveniles dispersed from their natal outcrop they later returned to the same outcrop. Similarly, in this study population only one of the five lizards that arrived during the study (breeding season only) and were genotyped was not significantly related to some current member of the population (LeBas unpubl. data). However, one individual per generation in such a small population is likely to be sufficient for panmixis (Wright 1931). Thus on the one hand there are indications that population differentiation may have occurred but also that there may be sufficient gene flow to prevent outbreeding depression in this species.

The restriction of the relationship between proportion of offspring surviving and mean  $d^2$  to females only, suggests the possibility of a maternal effect. In reptiles, maternal effects have been found to have a substantial influence on offspring phenotype (Shine and Downes 1999). Hence the interaction between offspring phenotype and survival will be the result of both the offspring's genotype and its maternal environment. Analysis of the influence of both female  $d^2$  and mean offspring  $d^2$  simultaneously on the proportion of a female's offspring that survived suggests there is a negative influence of the mother being outbred, separate from the offspring's own genotype. Females with genes common to the population may be better adapted to local conditions. Alternatively, outbreeding depression may be particularly manifest in the maternal environment.

#### *Alternative to Outbreeding Depression*

There is another potential explanation why outbred *C. ornatus* suffer a fitness cost. In the great tit, *Parus major*, egg hatching was negatively affected by inbreeding, but was more than compensated for by higher recruitment when either the male or female parent was inbred (van Noordwijk and Scharloo 1981). There was no evidence for inbreeding avoidance (van Noordwijk and Scharloo 1981). Van Noordwijk and Scharloo attributed their results to a few individuals produc-

ing a large proportion of the offspring in any one year such that offspring from these fitter individuals have an increased chance of mating with a relative. Hence fitter individuals are more likely to inbreed so that an indirect relationship between degree of outbreeding and fitness is established. In my study one male sired 26% of the offspring (LeBas 2001). In the small population studied these offspring will have a considerably higher chance of mating with a relative, and these same individuals are likely to have inherited their father's high fitness. Mating between fit individuals who also happen to be relatives can lead to the observed relationships of individuals that are less outbred having higher fitness. This study cannot distinguish between this hypothesis and that of outbreeding depression.

In conclusion, this study presents no evidence for mate choice based on genetic similarity, but instead reveals a cost to outbreeding. These findings are somewhat unexpected given the risk of inbreeding, but may not be counterintuitive given the population structure of this species.

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