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*Published in:*  
Royal Society of London. Proceedings B. Biological Sciences

*DOI:*  
[10.1098/rsbl.2003.0079](https://doi.org/10.1098/rsbl.2003.0079)

2003

[Link to publication](#)

*Citation for published version (APA):*  
Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B., & Wittsell, H. (2003). Major histocompatibility complex and mate choice in sand lizards. *Royal Society of London. Proceedings B. Biological Sciences*, 270(suppl. 2), S254-S256. <https://doi.org/10.1098/rsbl.2003.0079>

*Total number of authors:*  
6

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# Major histocompatibility complex and mate choice in sand lizards

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Recd 10.06.03; Acptd 08.07.03; Online 22.08.03

**In mice and man, females prefer males with a major histocompatibility complex (MHC) genotype different to their own. We tested whether this phenomenon also occurs in the Swedish sand lizard (*Lacerta agilis*). Females in a laboratory experiment preferred to associate with odour samples obtained from more distantly related males at the MHC class 1 loci. Data on free-ranging lizards suggest that associations between males and females are non-random with respect to MHC genotype. However, male spatial distribution and mobility during the mating season suggest that the non-random pairing process in the wild may also be driven by corresponding genetic benefits to males pairing with less related females.**

**Keywords:** major histocompatibility complex; mate choice; genetic complementarity; lizards

## 1. INTRODUCTION

Besides its immunological function, the major histocompatibility complex (MHC) has been recognized as a possible source of individual-specific body odours in several species of mammals, such as mice and man, and therefore implicated in mate choice (e.g. Penn & Potts 1999). We tested this possibility on the Swedish sand lizard (*Lacerta agilis*), a non-social species in which males provide nothing but genes to their partners or offspring (Olsson & Madsen 2001). Previous work on this species shows that males have overlapping home ranges that are on average ca. 1100 m<sup>2</sup>, mate repeatedly with a series of females, preferably large ones, and guard females for some time subsequent to the copulation (Olsson 1994). Male reproductive success is partly set by success in pre-copulatory mate acquisition, linked to male–male aggression and status signalling, and partly by post-copulatory processes (Olsson & Madsen 2001). Females have small home ranges (about a tenth that of males), are sedentary and seem to exert post-copulatory cryptic female choice, probably based on genetic complementarity of partners (Olsson *et al.* 1996).

Because olfactory cues may provide an important mechanism for individual recognition in lizards, we proposed that females might have the capacity to exert pre-copulatory mate choice based on MHC genotype. We tested this assumption in a staged laboratory experiment, in which females were allowed to associate with two males of different MHC genotype. We also conducted extensive fieldwork to evaluate any deviations from randomness of partners with respect to partner MHC relatedness.

## 2. MATERIAL AND METHODS

### (a) Laboratory study

Male and female Swedish sand lizards sampled in 1999 were artificially hibernated at the Department of Zoology, University of Gothenburg. Male hibernation was interrupted two weeks prior to that of females to allow time for spermatogenesis and production of the testosterone-dependent waxy secretions from the femoral pores (on the thighs). These secretions are produced during the breeding season and have been linked to territorial marking (Simon 1983), suggesting their utility for discriminating between rivals and, perhaps, partners. All females were sexually active and copulated in sperm competition experiments later in the same day as their MHC-odour trial.

Each odour preference trial was staged in a 650 mm × 300 mm × 350 mm glass aquarium, sterilized with 96% ethanol between trials, with clean sand as bottom substrate, and spotlights arranged symmetrically for basking. At each end, a sterile plastic ‘shelter’ box (165 mm × 115 mm × 30 mm) was placed, containing a non-perfumed cotton cosmetic swab (diameter of 35 mm), wiped across the femoral pores of a random male from the laboratory. A female was then placed in the middle of the cage and videotaped for 15 min. Half way through the trial series we turned the test cage 180° to ensure that no unidentified external factor directed the females and biased our results.

A blood sample was taken from *v. angularis* (upper jaw) and MHC class 1 haplotypes were determined by restriction fragment-length polymorphism (RFLP), using species-specific probes (Madsen *et al.* 2000). Band sharing between the female and her two potential ‘partners’ was calculated using the similarity index ( $2N_{AB}/(N_A + N_B)$ ) of Wetton *et al.* (1987), where  $N_{AB}$  represents the number of bands shared, and  $N_A + N_B$  the summed bands for the two individuals. The videotape was analysed for number of visits and total time spent on each side of the cage (and odour sample from the more- or less-related male), and number of visits to the shelter boxes.

### (b) Field study

The fieldwork followed a well-established routine that has been described in great detail elsewhere (e.g. Olsson 1994; Olsson & Madsen 2001). Therefore, only a brief account of procedures is given.

The field site at Asketunnan (50 km south of Gothenburg on the Swedish west coast) was visited every day that permitted lizard activity. The lizards were caught by noose or by hand, were measured (snout–vent length, total length, head length and width) and weighed (to the nearest 0.1 g). MHC class 1 haplotypes were determined as described above. Males may move more than 100 m a day (Olsson 1992), and we therefore assumed that any two males and females in the population were a potential sexual pair. We used a pairwise Pitman’s randomization test (available in STATXACT; Mehta & Patel 2000), to analyse the difference in band sharing between an observed pair contrasted against a hypothetical pair in which a new male was drawn at random for each female.

## 3. RESULTS

### (a) Laboratory study

The mean absolute number of male MHC class 1 RFLP fragments (referred to as ‘bands’) was 11.6 (± 2.2, s.d.) and differed on average by 3.3 bands between males in a trial (± 0.38,  $t = 8.74$ ,  $p < 0.0001$ ,  $n = 20$ ; excluding six trials in which both males had the same number of bands, all tests are pairwise  $t$ -tests). Females did not associate significantly more with the odours of males with more bands compared to males with less bands (mean difference in ‘most–least’ scent visits =  $-0.20 \pm 0.17$ , s.e.,  $t = 1.16$ ,  $p = 0.13$ ; number of visits in box  $-0.25 \pm 0.28$ ,

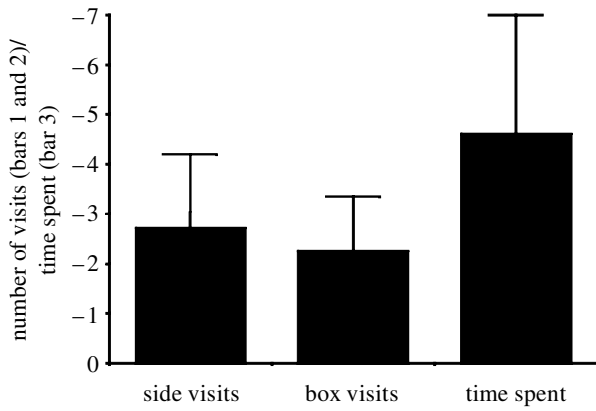


Figure 1. Differences in female spatial association between the two males ('most-least' related male; a more negative score thus represents more association with the least related male): difference in visits to side of cage  $-0.27 \pm 0.15$ , s.e.;  $t = 1.76$ ,  $p = 0.04$ ; in time allocation  $-226.5 \pm 107.8$  s, s.e.;  $t = 2.20$ ,  $p = 0.019$  in box visits  $-0.46 \pm 0.24$ ,  $t = 1.98$ ,  $p = 0.03$ ,  $n = 26$ ). To fit the three figures into the same panel, we converted the data by multiplying the raw data for number of side and box visits by 10, and time by 0.01.

s.e.,  $t = 0.89$ ,  $p = 0.19$ ; time difference  $-132.7 \pm 89.5$ , s.e.,  $t = 1.48$ ,  $p = 0.08$ ).

Males also differed significantly in genetic similarity ('band sharing') with the female. The average difference in band-sharing score between the males most and least related to the female was  $0.17 (\pm 0.02, \text{s.e.}, t = 8.36, p = 0.0001$ ; all tests are pairwise  $t$ -tests). There was no difference in male body size (difference in snout-vent length between most-least related male was  $0.3 \pm 1.5$  mm, s.e.,  $n = 26$ ;  $t = 0.22$ ,  $p = 0.83$ ). If sexually receptive females prefer to associate with a male more unrelated with respect to MHC, this predicts that females should pay more visits to, and spend more time in association with, the scent of the least related male. Indeed, this proved to be the case (figure 1; confirmed preference for the less related male expressed in scent visits, time spent per side and box visits). The number of visits into the shelter boxes showed the corresponding difference, being more prevalent in boxes with odour from less related males  $(-0.46 \pm 0.24, t = 1.98, p = 0.03, n = 26)$ .

#### (b) Field study

The Asketunna population shows a relatively high degree of band sharing, ranging between 0.63 and 1.00, and with a mean score of  $0.81 (\pm 0.11, \text{s.d.})$ . Theoretical pairs obtained by random sampling of a new partner for each female were more related than pairs observed in the wild (pooled data standardized by year, setting mean band sharing to zero and standard deviation to unity; Pitman's permutation test, exact probability,  $p = 0.032$ ; based on the laboratory results, we tested the one-tailed probability that observed pairs were less related than random pairs). Because in the wild males visit females and not vice versa, we suggested that male body size may influence the degree of band sharing in a pair so that large males (who can dominate small males) should prefer to associate with less related females. Indeed this proved to be the case. Male body mass was significantly negatively correlated with the

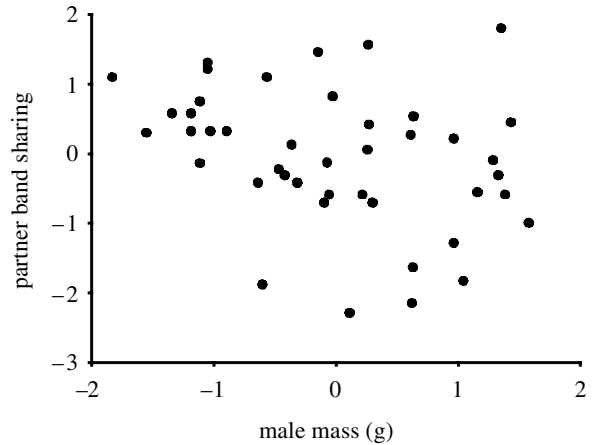


Figure 2. Male body mass is significantly negatively correlated with the band sharing of observed pairs (Spearman's rank order correlation coefficient,  $r_s = -0.31$ ,  $p = 0.038$ ,  $n = 45$ , data pooled over years after standardization of band sharing, mean set to zero and standard deviation to unity).

band sharing of observed pairs (figure 2; Spearman's rank order correlation coefficient,  $r_s = -0.31$ ,  $p = 0.038$ ,  $n = 45$ , data pooled over years after standardization of band sharing, mean set to zero and standard deviation to unity). The corresponding correlation between female mass and band-sharing score was, however, not significant ( $r_s = -0.20$ ,  $p = 0.19$ ,  $n = 46$ ).

#### 4. DISCUSSION

Our results largely agree with predictions from genetic complementarity theory (Zeh & Zeh 1996, 1997). In the laboratory study, females preferentially associated with male odour samples from the least related male. In support of this result, band sharing between observed pairs in the natural population was significantly lower than between pairs where the female was randomly re-assigned a new partner. Spatial biology in the wild, however, paints a more complex picture with males scent trailing sedentary females, not vice versa (Olsson & Madsen 2001). This makes it hard to envision how female choice could drive MHC-assortative pair formation in the wild. Male sand lizards, however, have been demonstrated to preferentially associate with large, more fecund females (Olsson 1993). Results from the present study demonstrate that large males prefer to associate with less related females, suggesting that male choice may drive the non-random pair formations. This is further supported by observations of males tongue flicking in a search for partners and rivals during the mating season, suggesting that males use scent to trail both partners and rivals. That this process involves odour identification related to the MHC is not known but is well established for mammals (Penn & Potts 1999). Female (and male) sand lizards could be associating with genes linked to the MHC, as seems to be the case in man, where genes for receptors within the olfactory epithelium are linked to HLA (i.e. MHC in man; Ziegler *et al.* 2000). Unfortunately, detailed genetic information of this kind is not available for any reptile species, and for very few species overall. Importantly, however, the behavioural pattern of our lizards observed in the laboratory agrees with the

work on mammalian models, for which the corresponding data we present from the wild is lacking. To the best of our knowledge, the current study is the first to show MHC-assortative pair formation in a natural population, although the proximate mechanism explaining this relationship is unclear.

Our study failed to confirm the relationship recently described in fishes (*Gasterosteus aculeatus*; Reush *et al.* 2001), where females preferred more MHC polymorphic males. However, since six trials had to be deleted because the males had the same number of bands, the power of detecting a 'number of alleles' effect was reduced compared to the trials where band sharing was assessed.

In conclusion, our results provide evidence that female sand lizards prefer odours of males with an MHC genotype different to their own. However, the lower than random MHC relatedness between males and females may be due to corresponding selective benefits for males pairing with less related females.

#### Acknowledgements

The authors are grateful for financial support from the Swedish Science Council and Collianders Stiftelse.

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