

## Article

# Experimental manipulation of size and shape of tail spots and sexual selection in barn swallows

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**Abstract**

Barn swallows *Hirundo rustica* have white spots on their tail feathers, and they have been hypothesized to be a handicap because white spots are prone to feather breakage, ectoparasites are disproportionately common in white spots, and size of white spots increases with tail length. Here I test for attractiveness of narrow and long tail spots by manipulation of their shape while using complete painting of spots and an absence of treatment as a control. Female barn swallows are known to differentially invest in reproduction when mated to attractive males. Spot manipulation took place during laying of the first clutch, and there were no effects of treatment on clutch size or brood size of first or second broods. In contrast, the incidence of second clutches and the total number of eggs and fledglings produced during the breeding season was larger in males with painting of the side of tail spots rather than painting of the tip of spots, painting of entire spots, or no treatment. These findings are consistent with the hypothesis that it is the shape rather than the size of tail spots that affects differential reproductive effort by female barn swallows.

**Key words:** *Hirundo rustica*, reliable signals, shape of tail spots, tail spots.

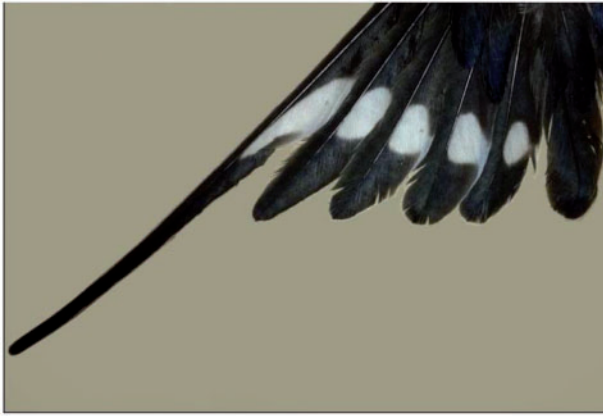
Spots and other markings on tail feathers of birds are common, with markings being present in nearly 80% of species in the Western Palearctic (Fitzpatrick 1998). Such spots have been shown to play a significant role in female mate choice, which is an important component of sexual selection (Höglund et al. 1990; Kose and Møller 1999; Kose et al. 1999; Hegyi et al. 2009; Penteriani and Delgado 2009; Griggio et al. 2011; Crowhurst et al. 2012). However, it remains unknown whether these experiments reveal a preference for larger spots or specific spot shapes. Finally, studies have indicated that white spots may play a role in dominance interactions through effects on immune response (Zanollo et al. 2012).

Barn swallows have a fork tail with tail feathers having white spots that decrease in size from the outer to the inner part of the tail (Figure 1). Males display the tail and its spots to females during courtship (Møller 1994). Tail spots are larger in adults than in juveniles, and they are larger in males than in females (Kose and Møller 1999). Tail length is positively correlated with size of tail spots (Kose and Møller 1999), whereas the size of tail spots is not

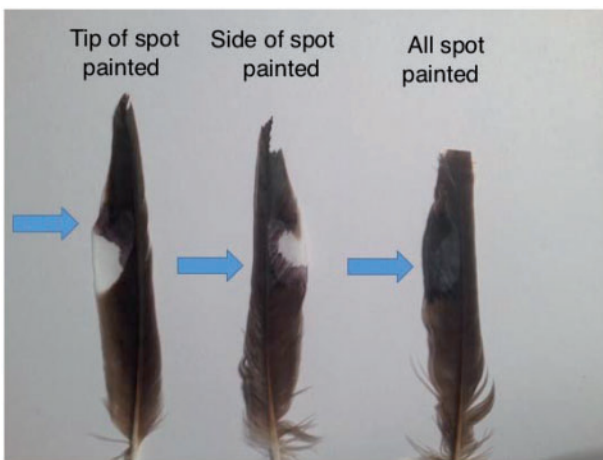
significantly correlated with the size of other morphological characters such as wing length, tarsus length, and body mass (Kose and Møller 1999). Spot size increased with age, spot size in year ( $i$ ) was positively correlated with spot size in year ( $i + 1$ ), and it increased among years with the variance in change of spot size being larger in males than in females (Kose and Møller 1999).

The interval between date of manipulation of tail spots with black paint and laying date increased for males with a large reduction in spot size (Kose and Møller 1999). Such a delay is costly because nestlings fledge later with consequences for delay in fall migration. Experimental painting of spot size reduced the size of first broods, the frequency of second broods, and the number of fledglings per season (Kose and Møller 1999; Kose et al. 1999). These effects were not caused by mate choice, but rather by differential parental allocation by females because all birds were already mated when the experiment was performed.

Saino et al. (2015) recently demonstrated that not only size, but also shape of tail spots affected components of sexual selection.



**Figure 1.** Tail feathers of a male barn swallow showing the location and the size of white tail spots.



**Figure 2.** Three tail feathers with painting of tip of spot, painting of side of spot, and painting of the entire spot as indicated with arrows.

These observations call for experimental verification of the link between spot shape and sexual selection. I used 4 experimental treatments to assess the effects of size and shape of tail spots (Figure 2): (1) painting of the sides of half of the tail spots; (2) painting the distal tips of half of the tail spots; (3) painting the entire tail spot; or (4) no treatment except capture and handling. Comparison of effects of treatments (1) and (2) would allow for a test of the importance of change in shape of tail spots, if tail spot size did not differ after treatment, whereas treatment (3) would allow for comparison of whether painting of the tips (2) had a similar effect as complete painting of tail spots (3). In contrast, comparison of effects of side removal (1) and complete painting of tail spots (2) would test for effects of size versus shape. I deliberately conducted the experiment after pair formation and when laying had started to allow for a similar baseline reproductive stage among the 4 treatments, but also in an attempt to avoid that attractive males acquired mates in better condition. Any later effects during the reproductive cycle would be due to subsequent effects of treatment of males on their reproductive decisions and those of their female mates. Because only females lay eggs, effects of treatment on timing of laying, clutch size, or incidence of second clutches should be due to experimental effects of treatment on females.

## Materials and Methods

### Capture of barn swallows

I captured 120 adult male and female barn swallows during May–June 2014 at Kraghede (57°12'N 10°00'E), Denmark, in a population that has been followed since 1971 (Møller 1994). A total of 50 randomly chosen males were used for the experiment, whereas the remaining 53 males were left as unmanipulated controls that would allow for monitoring the breeding population. All birds were captured in mist nets placed at open windows and doors, placed in cloth bags, provided with color rings, a numbered aluminum ring, and with color on the white breast plumage. They were subsequently measured, weighed, assessed for ectoparasites and tested for a number of behavioral responses to capture before being released in the same site as where captured. Not 1 single bird moved to a new location on capture or treatment.

### Experimental treatments

Captured males were randomly assigned to 1 of 4 treatments (Figure 2): 1) approximately half of the white spots were painted with a permanent marker “Pilot” with SCA-EF alcohol-based ink; 2) half of the distal tip of the white spots were painted with a permanent marker “Pilot” with SCA-EF alcohol-based ink; 3) all white tail spots were completely painted with a permanent marker “Pilot” with SCA-EF alcohol-based ink; or 4) no treatment. When the ink had dried after a few minutes, the bird was released. Previous experimental manipulation of tail spots has shown that the color of the painted spot is similar to that of the nearby black part of the feather (Kose and Møller 1999). A total of 17, 17, and 16 males were treated with the 3 treatments as described above.

### Reproductive information on treated individuals

I followed reproductive events by assigning males to nest sites using observations of color rings and marking on the breast feathers with binoculars throughout the breeding season. All nests were visited weekly and the content was recorded. If clutches had not been finished, this allowed for determination of laying date (1 = May 1), assuming that 1 egg is laid daily. If the clutch had been finished, I estimated laying date from clutch size, assuming that 1 egg was laid daily. Clutch size and brood size of first and second clutches, and the total number of eggs (number of eggs in first and second brood combined) and fledglings produced (number of fledglings in first and second brood combined) were recorded. Likewise, the presence or the absence of a second clutch was recorded. Unmated males were excluded from these analyses. There were no cases of re-laying due to predation.

### Statistics

Generalized linear model (GLM) were performed with normally distributed data and an identity link function (laying date), binomially distributed data and a logit link function (second brood or not), and Poisson distributed data with a log link function (all remaining variables). I report likelihood ratio tests, associated *P* values and estimates and standard error (SE). I used JMP (SAS Institute Inc 2012) in all analyses.

## Results

There were no significant differences in area of spots before experimental treatment ( $F=2.13$ ,  $df=3$ ,  $98$ ,  $P=0.10$ ). In contrast, the area of spots differed significantly among treatments after manipulation ( $F=531.24$ ,  $df=3$ ,  $98$ ,  $P<0.0001$ ). There was no significant

**Table 1.** GLMs of the relationship between reproductive variables of first (1) or second brood (2) and treatment of tail spots by removal of tip of tail spots, removal of side of tail spots, complete removal of tail spots from tail feathers or complete untreated control of barn swallows. GLMs were with normally distributed data with an identity link function (laying date), Poisson distributed with a log link function (clutch size, brood size, no. eggs (no. of eggs in first and second brood combined), no. of fledglings (no. of fledglings in first and second brood combined) or binomially distributed with a logit link function (presence or absence of a second brood)

Variable	$\chi^2$	df	P	Tip removal		Side removal		Complete removal		Mean control		
				Mean	SE	Mean	SE	Mean	SE	Mean	SE	
First brood												
Laying date	0.98	3, 99	0.81	27.24	2.61	24.38	1.83	26.47	2.38	25.32	1.32	
Clutch size	0.60	3, 99	0.90	5.06	0.13	5.13	0.13	4.59	0.19	4.96	0.09	
Brood size	4.43	3, 99	0.22	4.18	0.41	4.56	0.13	3.18	0.42	4.00	0.20	
N				17		16		17		53		
Second brood												
Presence/absence	10.97	3, 99	0.012	0.44	0.12	0.82	0.10	0.33	0.11	0.43	0.06	
Clutch size	0.51	3, 55	0.92	4.25	0.16	4.36	0.17	3.67	0.33	4.16	0.11	
Brood size	3.09	3, 55	0.38	2.88	0.67	4.36	0.17	3.67	0.33	3.81	0.22	
Total no. of eggs	10.61	3, 99	0.014	7.12	0.58	8.94	0.47	5.88	0.48	7.38	0.33	
Total no. of fledglings	21.13	3, 99	< 0.0001	5.59	0.71	8.38	0.43	4.47	0.61	6.23	0.39	
N				17		16		17		53		

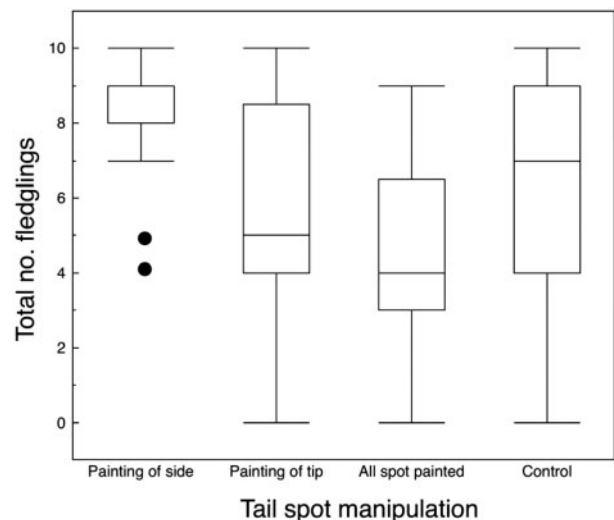
difference in area of spots after painting of the side and the tip of the spots ( $F = 1.03$ ,  $df = 1, 31$ ,  $P = 0.32$ ). Thus, the 2 treatments did not differ in area of spots, but rather in the shape of spots.

There was no significant bias in allocation of treatments as revealed by the nonsignificant effect of treatment on laying date of the first clutch of barn swallows (Table 1). Likewise, there was no significant difference in tail length among treatments ( $F = 1.91$ ,  $df = 3, 98$ ,  $P = 0.13$ ). Thus, the outcome of the experiment was not a consequence of nonrandom assignment to treatments. The main results of this experimental manipulation of size of white spots in the tail feathers were a significant effect on the incidence of a second brood, the total number of eggs per pair and particularly the total number of fledglings produced during the breeding season (Table 1; Figure 3). *Post hoc* tests of differences in the total number of fledglings revealed no significant differences between painting of tips and complete painting of the entire tail spot ( $\chi^2 = 2.12$ ,  $df = 1$ ,  $P = 0.15$ , estimate (SE) =  $-0.112$  (0.077)), whereas treatment of the side of tail spots increased reproductive success compared with the treatment of the tip of tail spots ( $\chi^2 = 9.25$ ,  $df = 1$ ,  $P < 0.0001$ , estimate (SE) =  $0.202$  (0.067); Table 1; Figure 3). The untreated control group and painting of the tip did not differ significantly ( $\chi^2 = 0.88$ ,  $df = 1$ ,  $P = 0.35$ , estimate (SE) =  $0.054$  (0.058)).

## Discussion

This tail spot manipulation experiment tested the hypothesis that not only the size, but also the shape of white spots on tail feathers of barn swallows affected female adjustment of reproductive decisions as reflected by the production of 2 broods per breeding season. Thus, painting of the tip of tail spots with a black permanent marker had a stronger negative effect on seasonal reproductive success as reflected by the number of fledglings than painting of the side of tail spots. Complete painting of spots with black color reduced reproductive success compared with painting of the side of spots, but not of the tips. In other words, painting of the tip of tail spots reduced reproductive success compared with painting of the side, implying that a pointy shape of tail spots was particularly attractive to females.

The present experimental study of tail spots did not significantly affect laying date, clutch size, or brood size of first brood, or clutch size or brood size of second brood. The effect of experimental treatment



**Figure 3.** Box plots of total number of fledgling barn swallows in relation to experimental manipulation of tail spots. The box plots show first and third quartiles, medians, quartiles, 5 and 95 percentiles, and extreme values. The median for painting of side was 9 fledglings.

was entirely due to the incidence of second broods as also found by Kose et al. (1999) and Kose and Møller (1999), although the latter also showed a weak effect on laying date. This effect of experimental treatment of tail spots on the incidence of second broods has consequences for the duration of the breeding season for individuals receiving different treatments and therefore also for the departure date for fall migration. Such delays may reduce subsequent survival prospects.

The present study is a consequence of effects of manipulation on males, females, or both. Previous manipulation of sexual attractiveness of males has shown that females invested differentially in offspring and in food provisioning when mated to a male that had become experimentally more attractive (de Lope and Møller 1993; see also Møller (1994) for similar results when male tails are not manipulated). We can infer that the manipulations in the present study negatively affected male food provisioning because female differentially invest in reproduction and food provisioning when mated

to an attractive male (de Lope and Møller 1993). Such an effect has also been shown in at least 3 species that provision (Burley 1986) or do not provision their offspring (Bluhm 1985; Yamamoto et al. 1989). I can only make inferences about the number of offspring produced in this experiment. Previous studies of barn swallows in Europe have consistently shown reduced frequency of extra-pair paternity in nests with attractive males, even when male tail length is experimentally manipulated (Møller and Tegelström 1997; Saino et al. 1997; Møller et al. 2003). Thus, there is little reason to assume that the findings reported here will be biased if extra-pair paternity was recorded.

Previous tail spot manipulation experiments on nominate barn swallows in Estonia have shown strong effects on the frequency of second broods and seasonal reproductive success (Kose and Møller 1999; Kose et al. 1999). These previous studies differ from the present study by painting of the side of the white spots on tail feathers (Kose and Møller 1999; Kose et al. 1999), whereas the present study manipulated either the side or the tip of the spots. Thus, we can infer from the present experiment that painting of the distal tip of the spots had a stronger negative effect on reproductive success than painting of the side of the white spots of the tail feathers. This implies that females are able to discern between painting of the side and the tip of the tail spots as reflected by a greater incidence of second broods produced by females mated to males with removal of the side of tail spots. All birds stayed at their once chosen breeding site for the second clutch, and second clutches were laid in the same nests as first clutches of a given pair.

A recent meta-analysis showed that there is selection for both larger tail spots and a larger circumference of tail spots (Saino et al. 2015; Romano et al. 2016) and that the strength of sexual selection on the size of tail spots differs among subspecies. Such population differences in female preferences for specific characters imply that different populations have diverged in phenotype and mate preference, perhaps as a consequence of incipient speciation.

Why should there be an effect of both spot size and spot shape on sexual selection? One possibility is that females perceive narrower and more pointed tail spots in males as reflecting longer tails during aerial displays. Indeed, experimental treatment in this study affected shape, but not size of tail spots, implying that the difference in the total number of fledglings is due to shape rather than size of spots. A potentially similar phenomenon was reported by Kelley and Endler (2012), who showed an effect of size–distance gradients of objects on the courts of bowerbirds *Ptilonorhynchus nuchalis* caused by perceived size of displays. It is important to note that barn swallow males spread their tails during aerial displays, when followed by a potential female partner (Møller 1994), but also whenever barn swallows spread their tails such as at tight turns. These aerial displays occur throughout the breeding season.

The findings reported here have evolutionary implications. Although the present study of barn swallows showed effects of size and shape of white tail spots on seasonal production of fledglings, it is known that populations of barn swallows differ in effect of tail spots on the intensity of sexual selection (Romano et al. 2016). Such differences in mate preference should lead to divergence in phenotype among populations. Analysis in progress of the size of white tail spots in males and females from a number of breeding populations across Europe and Asia will help determine the relationship between spot size and shape on one hand and tail length on the other. In particular, differences in size and shape of tail spots among populations may reflect differences in the role of tail length and size and shape of tail spots for phenotypic divergence and hence speciation.

In conclusion, female barn swallows differ in their response to experimental manipulation of the size and the shape of white spots in the tail feathers of males. These findings imply that pointed white spots in the tail feathers of male barn swallows are more attractive to females.

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