

**Sexual Selection on Multiple  
Ornaments in the Barn Swallow**  
*Hirundo rustica gutturalis*

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# **Sexual Selection on Multiple Ornaments in the Barn Swallow**

***Hirundo rustica gutturalis***

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# ABSTRACT

About 150 years ago, Charles Darwin proposed a concept “sexual selection” for explaining elaborate male ornamentation, which seemed to have rather negative influence on the survivorship. Thereafter, many studies have successfully supported the existence of sexual selection on a single male ornament. However, animals developed more than a single male ornament, and the reason of such multiple ornaments remains unclear. The barn swallow *Hirundo rustica gutturalis* was used to study on the existence of two male ornaments, red throat coloration and white spots in the tail. The former was found to fade with time after molt and the methods correcting for plumage color fading was established. In the study population located at Joetsu city in Japan, extra-pair paternity was rare. Males with colorful throat and large white spots in the tail had the mating advantages in terms of early breeding onset. Both ornaments might be used in sexual selection either directly through the ornaments, i.e. intersexual selection, or indirectly through the territory they hold, i.e. intrasexual selection. Females chose their mates according to the territory quality, indicating the existence of intrasexual selection. Each selection on male ornaments was determined by examining the relationships among male ornaments, territory quality, and breeding onset date. Males with colorful throat held high-quality territory, suggesting intrasexual selection, while males with large white spots had the mating advantages in terms of early breeding onset after controlling for territory quality, suggesting intersexual selection. These results in the barn swallow support the multiple receiver hypothesis, which predicts that one ornament is intended for females and the other ornament is used in male-male competition.

**Keywords:** barn swallow; coloration; female choice; *Hirundo rustica gutturalis*; multiple ornaments; paternity; sexual selection; territory quality

# GENERAL INTRODUCTION

In the letter penned to an American botanist Asa Gray, Charles Darwin wrote: ‘The sight of a feather in a peacock’s tail, whenever I gaze at it, makes me sick!’ (Letter to Asa Gray, 3 April 1860). To explain these elaborate male “ornaments” which have some viability disadvantage, he proposed a concept of sexual selection, or of ‘the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction’ (Darwin, 1871). According to Darwin, male ornaments evolve through sexual selection by female choice (i.e. intersexual selection), or through sexual selection by contests over females (i.e. intrasexual selection), as reviewed by Andersson (1994). Thereafter, many studies have successfully supported the sexual selection processes (Hill, 2006; Senar, 2006; Brooks and Griffith, 2010).

Most studies on sexual selection have focused on a single male ornament, though animals have more than a single male ornament (Møller and Pomiankowski, 1993). For example, even a single feather in a peacock’s train, which may appear to be a single ornament to the human observers, may in fact convey a multitude of potentially independent signals, encoded by the length of the tail, the number of ocelli, brightness, and so on. In addition, a peacock has other ornaments including an iridescent blue ventral color, an iridescent green dorsal color, white facial skin patches and an exaggerated crest (Møller and Petrie, 2002). Despite the numerous examples of complex male ornamentation in animals, the reason for such complexity remains unclear.

The question why animals developed multiple ornaments is intriguing, because signaling is often associated with considerable costs from time and energy loss as well as

predation and disease risk (Bradbury and Vehrencamp, 1998). Several hypotheses have been proposed to overcome such constraints, including obsolete signals, i.e. ornaments for which female preference has been lost but which are maintained because they are not costly (Møller and Pomiankowski, 1993), multiple message, i.e. ornaments subject to female mating preferences because of reflecting different aspects of the quality of the male (van Doorn and Weissing, 2004), dynamic female choice, i.e. fluctuations in ecological and social environments can maintain female preference for multiple male ornaments (Bro-Jørgensen, 2010), and multiple receiver hypothesis, i.e. some ornaments are used in intersexual selection and the other ornaments are used in intrasexual selection (Andersson et al., 2002).

According to van Doorn and Weissing (2004), accurate classification of hypothesis needs to determine sexual selection on each ornament through female choice and male-male competition, respectively. When females choose multiple ornaments, the relationship between each ornament and male quality should also be determined.

The barn swallow *Hirundo rustica* is a suitable species for studying on multiple ornaments. Two conspicuous male ornaments are found: a red throat patch and white spots in the tail (Fig. 1). The former has been demonstrated to be sexually selected in some populations (Ninni, 2003; Safran and McGraw, 2004), and the latter in other populations (Kose and Møller, 1999; Kose et al., 1999). However, it is still unknown whether and how the two ornaments are sexually selected in a single population.

Tail length is another candidate of target in sexual selection for barn swallows. Although male long tail is sexually selected in some populations (Møller, 1994), it would be naturally selected because of aerodynamic efficiency and flight manoeuvrability (Norberg,

1994; Buchanan and Evans, 2000; Cuervo and Ayala, 2005), and the length can be also explained by natural selection (Evans, 1998; Rowe et al., 2001; Bro-Jørgensen et al., 2007). Therefore, in the present study, tail length was not included as an ornament that is difficult to be explained with survival advantage.

In the study population located at Joetsu city in Japan, both throat coloration and white spots in the tail are conspicuous and seem to be difficult to be explained with survival advantage, inspiring the existence of sexual selection. Sexual selection in this monogamous species can be studied by investigating extra-pair paternity and mating advantage in terms of early breeding onset in relation to male ornaments (Møller, 1994). Males may attract females directly through their ornaments or indirectly through the territory they hold. Understanding the relative importance of the direct and indirect effects of male ornaments on male mating advantages indicates the relative importance of intersexual and intrasexual selection on each male ornament (Bart and Earnst, 1999). Bart and Earnst (1999) states that intersexual selection and intrasexual selection could be decoupled by focusing on territory quality of the swallows. Although studies on sexual selection have exclusively been reported on female choice in the barn swallow (Turner, 2006), male-male competition should be studied for the comprehensive understanding of sexual selection.

The present study investigated the reason why animals have multiple ornaments. The seasonal change of plumage coloration was determined (Chapter 1). Since individuals had been captured at different times during the breeding season, these effects were taken into account in the present study. Through DNA fingerprinting method, the frequency of extra-pair paternity and its influence on sexual selection were described (Chapter 2). Sexual selection on

each ornament was examined (Chapter 3). Female mate choice was studied in relation to territory quality (Chapter 4). Then, intrasexual and intersexual selection on multiple male ornaments was investigated by statistically controlling for territory quality (Chapter 5).

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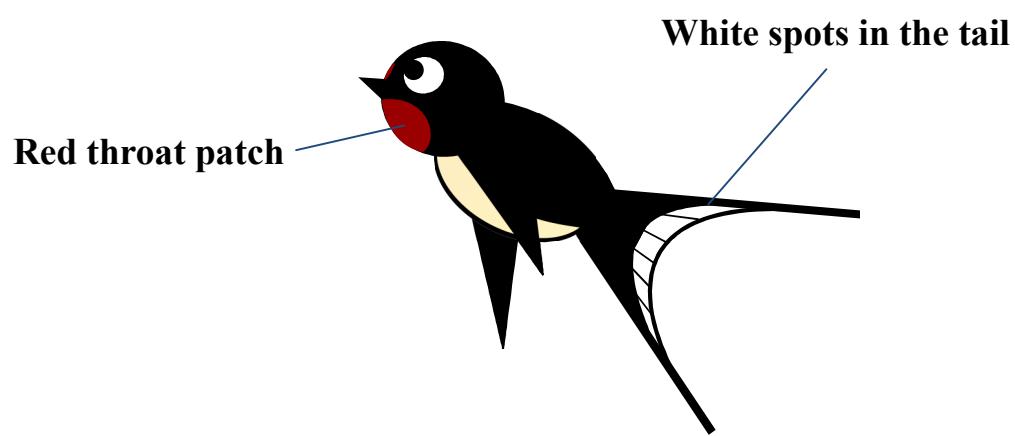
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**Figure 1.** A male barn swallow showing the two ornaments, red throat patch and white spots in the tail.

**CHAPTER 1**

**SEASONAL CHANGE OF PLUMAGE COLORATION IN**

**THE BARN SWALLOW\***

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## **ABSTRACT**

It is necessary to correct plumage color fading when comparing the plumage coloration of birds captured at different times during the breeding season. I proposed two methods for correcting plumage color fading and compared them using the throat feathers of the barn swallow (*Hirundo rustica gutturalis*). One method uses the color shift of feathers on live birds during the same breeding season (the field method). The other method uses longitudinal color measurements of feathers placed on the open ground (the experimental method). This method has an advantage of not capturing the birds twice. The field and experimental methods estimated similar rates of fading of hue and brightness. Saturation was predicted to fade with time in the experimental method, which is unrealistic because there was no effect of time on saturation in live birds. Using the field method, I derived an equation for correcting plumage color fading in barn swallows. When assessing changes in plumage color, the field method should be used whenever possible since this method estimates plumage color fading in live birds.

**Keywords:** barn swallow; bird coloration; plumage color fading

## INTRODUCTION

Evolutionary biologists have long been interested in the evolution of brightly colored plumage (Hill and McGraw, 2006a, b). Although the plumage coloration of birds has been recognized as a fixed trait once the bird has molted, it was recently demonstrated that feather color fades after molting as a result of UV damage and abrasion (Montgomerie, 2006; McGraw and Hill, 2004; Figuerola and Senar, 2005). This was because it is only during the past few years that objective methods quantifying plumage coloration has become established (Hill and McGraw, 2006a). Feather color fading predicts that early breeders have more colorful plumage than late breeders, when early breeders are captured earlier than late breeders, which is often the case in practice. This is the same prediction made by sexual selection theory, which states that colorful birds breed early in the season (Kirkpatrick et al., 1990; Hill, 2002). Therefore, I should take into account the effect of time on plumage coloration, particularly when comparing individuals captured at different times during the breeding season.

When comparing the plumage color in a bird species, one approach is to capture all individuals at approximately the same time (McGraw and Hill, 2004). This approach can diminish seasonal color difference between birds made by feather color fading. However, for some bird species, such an approach is not possible due to the technical difficulty. Here, I propose an alternative approach, which is to correct for the effect of time on plumage coloration. To date, this approach has not been attempted with any bird species in this young field of research. I examined two methods designed to correct for plumage color fading. One of these methods uses a shift of feather color on live birds during the same breeding season (hereafter the field method). The other method uses longitudinal color measurements of

feathers placed on the open ground (hereafter the experimental method). This method has an advantage of not capturing the birds twice. I compared these two methods using the throat feathers of the barn swallow (*Hirundo rustica gutturalis*), a model species in sexual selection (Møller, 1994). The red throat coloration of this species is known to be a sexually selected trait, because colorful males breed earlier than others (Safran and McGraw, 2004), and color-enhanced males have higher within pair paternity (Safran et al., 2005). It is also known that swallows use phaeo-melanin to color their throats (McGraw et al., 2004). However, no studies have investigated whether the throat coloration fades with time. Therefore, I first confirmed the existence of feather color fading and then tested the two correction methods. I also studied the influences of other conditions, such as sex, year, and season, on feather color change because these may also have an influence on feather color fading (Figuerola and Senar, 2005; Safran et al., 2005).

## MATERIAL AND METHODS

### General procedures

The present study was conducted in 2006 and 2007 in a residential area of Joetsu City, Niigata Prefecture, Japan (see Tajima and Nakamura, 2003). Birds were captured by hand-net and color-ringed. The sex of an individual was determined by the presence (female) or absence (male) of an incubation patch. I collected 5–10 throat feathers from each bird.

Once in the laboratory, I piled five feathers on a piece of white paper so that there were five layers at the distal end of the feathers. The feather samples were scanned at 800 dpi resolution using an EPSON GT 9300 UF scanner, and the images obtained were imported into

the Adobe Photoshop Elements 3.0 program (Adobe Systems, USA). I used a fixed number of feathers to measure plumage coloration since the number of feathers strongly influences the values of plumage color variables (Quesada and Senar, 2006). I measured the mean RGB values in a square of 30 pixels near the distal end of the feather sample. The mean RGB values were converted to hue-saturation-brightness (HSB) values using the algorithm described in Foley and Van Dam (1984). Hue (H) has normally been the parameter of interest when studying bird coloration (Hill and McGraw, 2006b). However, because the focus of this study was to correct totally for plumage color fading, I also examined the effect of time on saturation (S) and brightness (B) as well as hue. The repeatability of these variables (Lessells and Boag, 1987) was highly significant and had medium to high values (from the whole populations in 2006 [males, H:  $r$  (repeatability) = 0.81,  $F_{1,42} = 9.42$ ,  $P < 0.001$ ; S:  $r = 0.80$ ,  $F_{1,42} = 9.04$ ,  $P < 0.001$ ; B:  $r = 0.90$ ,  $F_{1,42} = 18.82$ ,  $P < 0.001$ ; females, H:  $r = 0.89$ ,  $F_{1,28} = 17.11$ ,  $P < 0.001$ ; S:  $r = 0.65$ ,  $F_{1,28} = 4.69$ ,  $P < 0.001$ ; B:  $r = 0.91$ ,  $F_{1,28} = 22.07$ ,  $P < 0.001$ ]). These color variables quantify visible light only which is sufficient for this species because the throat feathers of barn swallows do not exhibit any UV-reflectance peak (Safran and McGraw, 2004).

### **Field method**

I used birds captured twice in the same breeding season ( $N = 32$  individuals in 2006 and  $N = 33$  in 2007). I compared the color variables (i.e., H, S, and B) of the initial and final scores to ascertain the existence of a color fading effect in the throat feathers of this species. I subtracted the initial from the final scores to compute the seasonal shift in plumage color. The median

interval between these scores was 50 (range 6–73) in 2006 and 51 (range 9–86) in 2007.

## **Experimental method**

The field experiment was carried out in 2007. I used samples collected in both 2006 and 2007 ( $N = 10$  in 2006 and  $N = 7$  in 2007). I kept the samples in the dark prior to the experiment because long-term feather storage in the dark does not change the pigment composition of the feather samples (McGraw et al., 2004). In this experiment, I used a transparent plastic mount instead of white paper because paper cannot withstand rain in the field. Because of the pale mount coloration, the same samples gave smaller saturation and larger brightness values in the experimental method than in the field method. This difference, however, did not influence the seasonal color shift of the samples because I used the same type of mount for each set of measurements. I placed feather samples on the underside of dummy birds that were made of wood 30 cm in length and 3 cm square in cross-section. I used one sample per dummy bird (total, 17 dummy birds), each of which was placed about 120 cm above an area of open ground (see Fig. 1). I started the experiments at the beginning of April when swallows start to arrive at my study site. The color of each sample was measured at the start of the experiment and once a week during the subsequent seven weeks.

## **Statistical analysis**

I used a paired *t*-test and a two-sample *t*-test to examine within-season temporal changes in plumage coloration. To more accurately predict the effect of time on color change, I used a generalized linear model with a normal error and identity link function to examine the

relationship between color change and the interval between measurements. The intercepts were fixed at zero in this analysis, since there should be no color shifts when the interval is zero. I included the interaction terms between the interval and conditions (sex, date, and year) in the initial model. In the experimental method, I used a linear mixed model (R statistical environment, function ‘LME’), which included individual feather samples as a random effect to account for repeated measures. Because the model accounts for a subject-specific random component of the residual, I can statistically control for the initial color difference among individual feather samples. I included the interaction terms between time and conditions (sex and year) in the initial model. To explore the relationship between time and color shift in each model, I used locally weighted scatter plot smoothers (LOWESS curves; cf. Safran and McGraw, 2004). These LOWESS curves enabled me to determine whether linear or other functions, such as quadratic or exponential functions, would be suitable for modeling the relationship between two variables. All explanatory terms were initially entered into the model. The fitted model included only significant terms. In the tables presented in this paper, the statistics and *P* values of significant terms are derived from the fitted model, whereas the statistics and *P* values of non-significant terms are from the main term and each interaction term added separately. To compare the field and experimental methods, the color fading rate per day was estimated from the fitted model in each method. The color fading rate per day was estimated from the fading rate per week divided by 7 in the experimental method. All data analyses were performed using the R (version 2.8.0) statistical package (R Development Core Team, 2008).

## RESULTS

### Effect of color fading on each variable

To ascertain the existence of a color fading effect in the throat feathers of this species in the field, I first compared the color variables of the initial and final color scores. Throat coloration in barn swallows exhibited a significant increase in hue (less red) and in brightness (paler coloration), but no change in saturation over the season (Fig. 2). There was also a significant increase in hue and brightness, but no change in saturation, when I compared the mean color variables between the initial and final scores (two-sample *t*-test, Hue:  $t = -2.30$ ,  $P = 0.02$ ; Saturation:  $t = -0.70$ ;  $P = 0.49$ ; Brightness:  $t = -2.19$ ,  $P = 0.03$ ).

### Field method

Hue and brightness shifts were explained only by the interval between measurements (Table 1). These variables faded linearly with time (Fig. 3). There was no significant effect of the interaction between the interval and the three conditions (sex, year, and initial capture date). The saturation shift was not explained by any term (the main effect of the interval or its interaction with sex, year, and initial capture date; see Table 1).

### Experimental method

All color variables were significantly explained only by time (Table 2). Although hue and brightness faded linearly with time, saturation faded curvilinearly (Fig. 4). For all three color variables, there was no significant effect of the interaction between time and the conditions (sex and year; Table 2).

### **Comparing the estimates from the two methods**

The two methods predicted similar rates of hue and brightness fading (Table 3). In contrast, for saturation, the two methods yielded different predictions. Although the field method predicted no significant effect of time on saturation, the experimental method predicted a significant effect of time on saturation in a curvilinear form.

## **DISCUSSION**

Hue and brightness faded within individuals as well as at the population level over the season (Fig. 2). Although the measured effect of time on coloration was highly variable among birds, some seasonal trends were observed (Fig. 3). A method is needed for removing the effect of feather color fading in this species so as not to confuse this effect with other effects (e.g. age and/or attractiveness at mating). Both correction methods predicted that hue and brightness fade linearly with time (Figs. 3 and 4). Although year, season, and sex have been predicted to affect the rate of feather color fading (Delhey et al., 2006; Safran et al., 2006), these factors did not influence the color fading rate in the present study (Tables 1 and 2). Therefore, I can correct hue and brightness only with time in barn swallows.

Saturation can be used without correcting the effect of time in this species because this variable had no consistent effect over time (Figs. 2 and 3). On the other hand, the experimental method predicted that saturation fades with time (Fig. 4). This might be due to the difficulty of simulating field conditions using the experimental method. Specifically, it is not possible to take bird behavior into account using this method, unlike the field method that directly measured the seasonal color change in birds. It has been demonstrated that birds can

control their plumage coloration by behavior and the use of preening oil (Montgomerie et al., 2001; Surmacki and Nowakowski, 2007). Another explanation is that I cannot detect complex patterns of saturation fading using the field method. However, this is improbable, because I included three interaction terms, and there was still no significant effect of time (Table 1).

Using the field method, I devised the following equation to correct plumage color fading in the throat feathers of the barn swallow:

$$\text{Corrected (H, S, B)} = \text{measured (H, S, B)} - (0.023, 0, 0.097) \times \text{Days}$$

Here, H, S, and B represent hue, saturation, and brightness, respectively. The term “Days” indicates the days elapsed from the capture date of the first bird to that of each bird.

One of the requirements for using the field method is that it is necessary to capture birds twice each season. If this cannot be achieved, then it is necessary to use the experimental method for correcting seasonal color change. As mentioned in the second paragraph of the discussion, this method might fail to estimate actual feather color fading in the field. Therefore, if it is necessary to use the experimental method, careful consideration would need to be given to the estimates obtained using this method.

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**Table 1.** Generalized linear model showing the effect of terms on color shift with the intercepts fixed to zero using the field method.

	Model terms	df	effects (SE)	F	P
Hue shift	Interval	1	0.023 (0.004)	29.77	<0.0001
	Interval × Sex	1	—	0.32	0.58
	Interval × Year	1	—	0.73	0.39
	Interval × Date	1	—	0.00	0.96
Saturation shift	Interval	1	—	1.46	0.23
	Interval × Sex	1	—	0.00	0.98
	Interval × Year	1	—	0.34	0.56
	Interval × Date	1	—	0.02	0.90
Brightness shift	Interval	1	0.097 (0.013)	26.28	<0.0001
	Interval × Sex	1	—	0.02	0.89
	Interval × Year	1	—	0.01	0.91
	Interval × Date	1	—	0.13	0.72

I used a total of 65 samples ( $N_{\delta 2006} = 18$ ,  $N_{\varphi 2006} = 14$ ,  $N_{\delta 2007} = 18$ ,  $N_{\varphi 2007} = 15$ ). The median date of the initial scoring was 21 May (range 28 April–12 June) in 2006, and 22 April (range 28 March–31 May) in 2007.

**Table 2.** Generalized linear mixed model showing the effects of terms on color shift using the experimental method.

Model terms		df	effect (SE)	F	P
Hue	Time	1	0.17 (0.03)	39.12	<0.0001
	Time × Sex	1	—	0.05	0.82
	Time × Year	1	—	0.76	0.39
Saturation	log (Time)	1	2.50 (0.36)	47.14	<0.0001
	log (Time) × Sex	1	—	0.98	0.33
	log (Time) × Year	1	—	0.94	0.33
Brightness	Time	1	0.68 (0.11)	41.72	<0.0001
	Time × Sex	1	—	0.05	0.82
	Time × Year	1	—	0.93	0.34

Individuals were treated as random effects.

I used a total of 17 samples ( $N_{♂2006} = 6$ ,  $N_{♀2006} = 4$ ,  $N_{♂2007} = 6$ ,  $N_{♀2007} = 1$ ).

**Table 3.** Feather color fading rates estimated using the two methods.

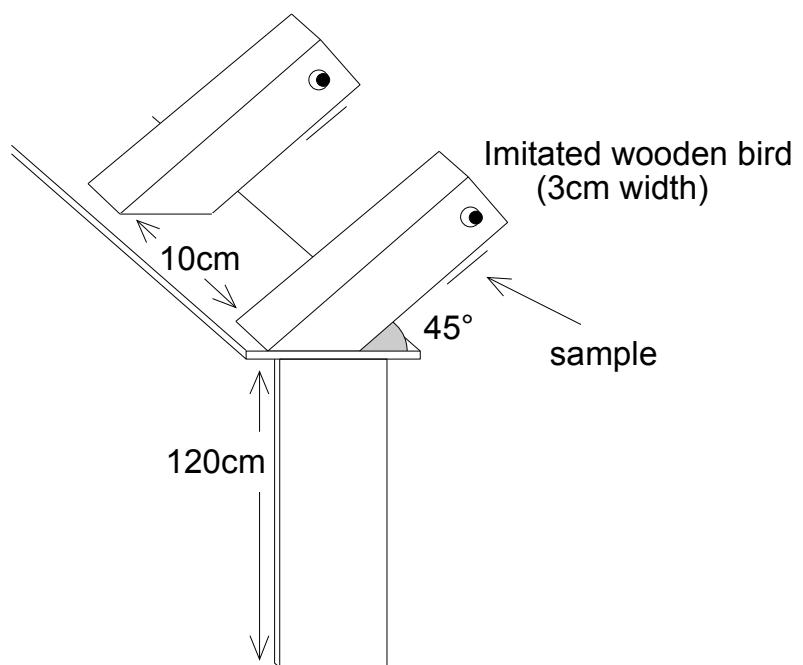
Color variables	Field method (65)	Experimental method (17) <sup>a</sup>
Hue (degree/day)	0.023 ± 0.004	0.024 ± 0.004
Saturation (percent/day)	0 <sup>b</sup>	— <sup>c</sup>
Brightness (percent/day)	0.097 ± 0.013	0.098 ± 0.015

Numbers in parentheses indicate sample sizes.

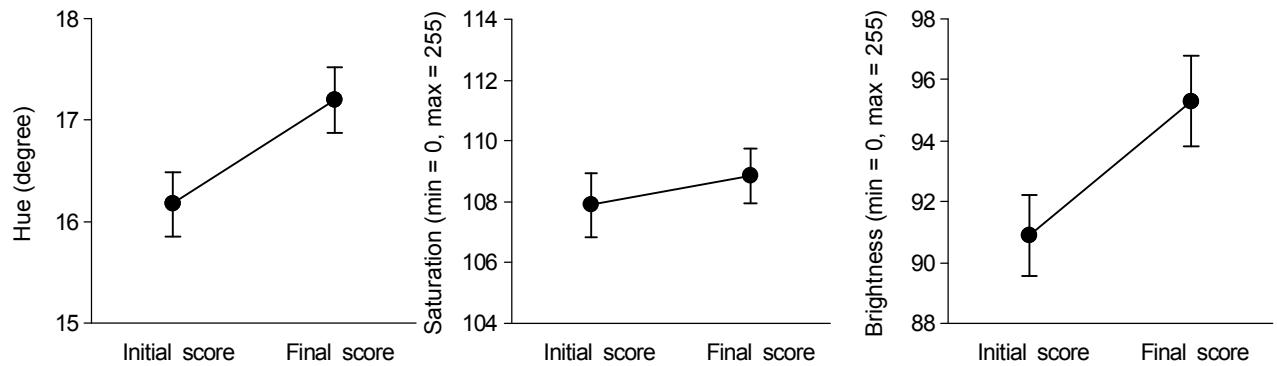
<sup>a</sup> Estimates are calculated by the feather fading rate per week divided by 7.

<sup>b</sup> There is no significant effect of time on this variable.

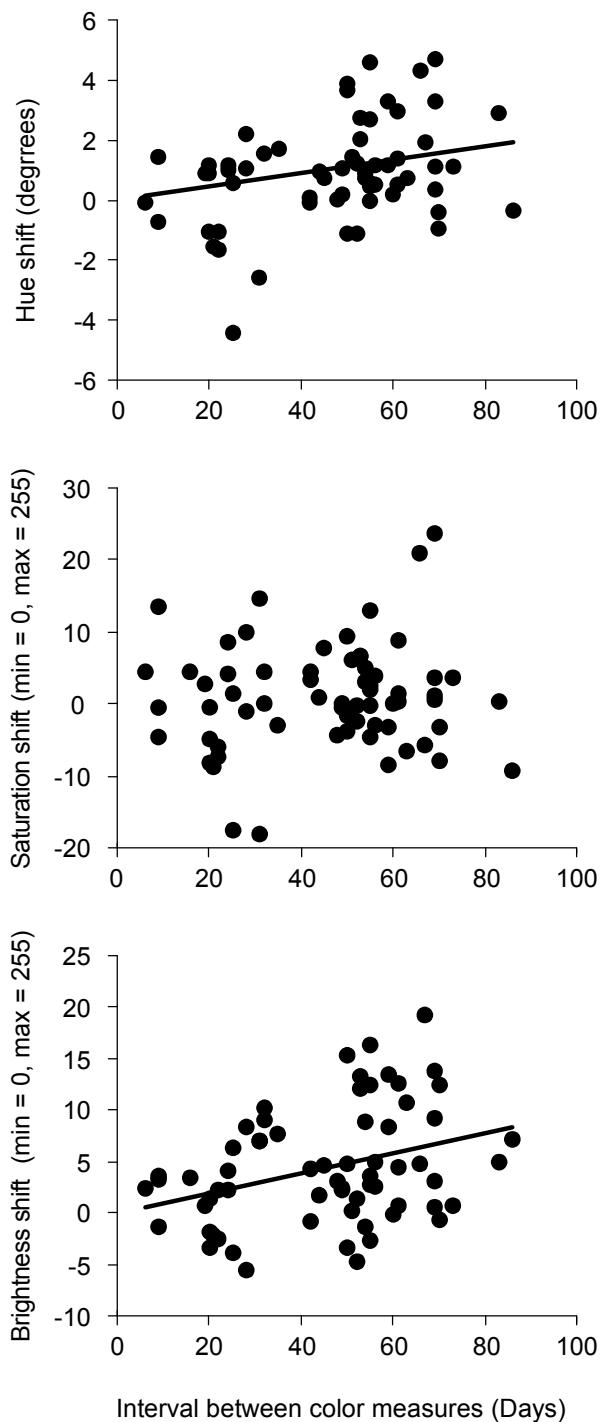
<sup>c</sup> I was unable to estimate the fading rate because of the curvilinearity.



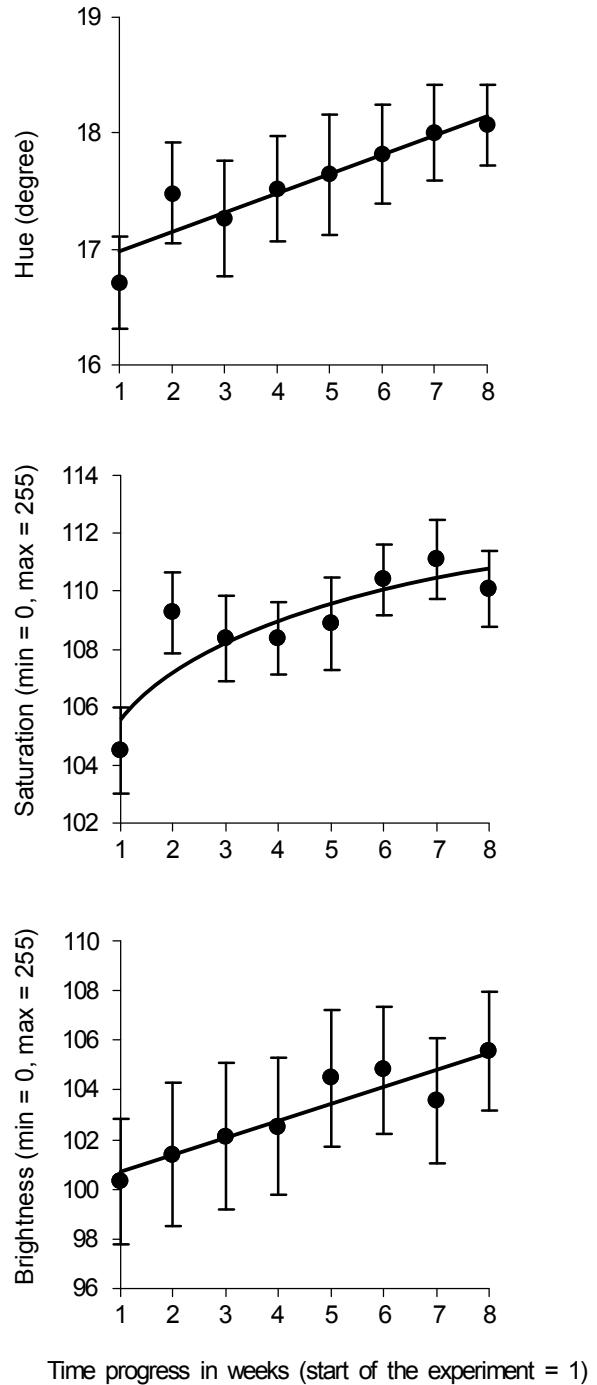
**Figure 1.** Sketch of the dummy bird. The feather sample is attached to the throat part.



**Figure 2.** Comparison of the initial and final color scores (mean  $\pm$  SE). Hue:  $t = -4.87$ ,  $P < 0.0001$ ; Saturation:  $t = -1.05$ ,  $P = 0.30$ ; Brightness:  $t = -6.34$ ,  $P < 0.0001$ . I used data from 65 samples collected over two years ( $N_{\text{♂}2006} = 18$ ,  $N_{\text{♀}2006} = 14$ ,  $N_{\text{♂}2007} = 18$ ,  $N_{\text{♀}2007} = 15$ ).



**Figure 3.** Relationship between plumage hue (upper), saturation (middle), and brightness (bottom), and the interval between color measurements in the field. Higher hue, saturation, and brightness scores indicate that the color faded to a greater extent. Regression lines are from the fitted model using a generalized linear model with the intercept fixed at zero (Table 2).



**Figure 4.** Relationship between plumage hue (upper), saturation (middle), and brightness (bottom), and the weeks since the start of the experiment. Higher hue, saturation, and brightness scores indicate that the color faded to a greater extent. Regression lines are from the fitted model using a generalized linear mixed model (Table 3). Intercepts, which were also predicted from the models, were as follows: 16.82 for hue, 105.56 for saturation, and 100.00 for brightness. Bars depict  $\pm$  SE.

**CHAPTER 2**

**LOW LEVEL OF EXTRA-PAIR PATERNITY IN A  
POPULATION OF THE BARN SWALLOW *Hirundo  
rustica gutturalis*\***

\*Accepted by Ornithological Science, 9:161–164 (2010)

## **ABSTRACT**

On average, male birds other than social father sire more than 10% of all offspring in bird species. Levels of extra-pair paternity below 5% of offspring are rarely found and are now considered worthy of explanation in monogamous birds. I recorded the lowest levels of paternity loss ever reported in a population of the barn swallows *Hirundo rustica*. The levels of extra-pair paternity were below 5% of offspring (7/243 in 2005 and 1/53 in 2006). I discuss my results in relation to the density-dependence of extra-pair paternity.

**Keywords:** density-dependence; extra-pair young; *Hirundo rustica*

## INTRODUCTION

Most socially monogamous birds are in reality not genetically monogamous due to extra-pair paternity (Griffith et al., 2002). On average, males other than the social father sire more than 10% of all offspring (Griffith et al., 2002). The occurrence of extra-pair paternity can be explained by the advantage of multiple mating at least for males (Andersson, 1994; Griffith et al., 2002; Arnqvist and Kirkpatrick, 2005). Low levels of extra-pair paternity (< 5%) in monogamous birds are rarely found, and thus, are now considered worthy of explanation (e.g. Griffith et al., 1999; Robertson et al., 2001; Griffith et al., 2002).

The barn swallow *Hirundo rustica* is a monogamous bird and a famous model species used to study extra-pair paternity (reviewed in Møller, 1994; Turner, 2006). Several studies have described the occurrence of extra-pair paternity (or its cause, extra-pair copulation) in relation to male traits (e.g. tail length: Møller and Tegelström, 1997; plumage coloration: Safran et al., 2005; body condition: Kojima et al., 2009), male behaviour (e.g. mate guarding: Møller, 1994), genetic compatibility (Kleven et al., 2005), and other parameters related to population dynamics (breeding density: Møller, 1994; breeding synchrony: Saino et al., 1999; population size: Safran, 2007; reviewed in Turner, 2006). Despite these exhaustive studies of extra-pair paternity in several populations, extra-pair paternity reported is consistently high in this species (about 20%: reviewed in Turner, 2006), especially in studies with ample sample size (>200: cf. Griffith et al., 2002). Here, I report the lowest level of paternity loss known in a population of barn swallows with ample sample size (total 296 nestlings), and discuss a possible explanation.

## MATERIAL AND METHODS

The field study was carried out in 2005 and 2006 in a residential area of Joetsu City, Niigata Prefecture, Japan ( $37^{\circ}07' N$ ,  $138^{\circ}15' E$ ). Barn swallows *H. r. gutturalis* nest here under the eaves of a covered sidewalk along the street and breed in a loose colony in this area (see Tajima and Nakamura, 2003). I inspected the nests every other day to record breeding events. This allowed determination of: (1) the laying date, which was expressed as the date on which the first egg of the first clutch was laid, and (2) clutch size. Laying date was estimated by backdating one egg per day from the date of the first record of eggs in the nest. To determine the hatching date, I inspected nests every day around the estimated hatching date (10 days after initiation of incubation).

Following the method used by Saino et al. (1999), I plotted all nests found in the study site on a map and measured the distance between the focal nest and the nearest asynchronous male (i.e. a neighbour whose mate laid their first egg more than eight days before or six days after the mate of the focal male). Only asynchronous males were used for the analyses, because males intensely guard their mates during the fertile period of their mates, and, at least in European subspecies, all cuckoldry happens during this period (Saino et al., 1999). Although Saino et al. (1999) averaged the distance to the two nearest males, I used the distance to a nearest male, because of the high variability in the distance between the nests in my study site.

Adult swallows were captured in sweep nets while roosting at night. The birds were provided with a numbered aluminum ring provided by the Ministry of the Environment, Japan and an individual combination of two or three half-sized colour rings, which were made by

splitting plastic rings (AC Hughes, Middlesex). Sex was determined by the presence (female) or absence (male) of an incubation patch, as only females have a brood patch in this species (Turner, 2006). Nest ownership was subsequently determined with binoculars.

At capture, I obtained a small sample of blood from the brachial vein. The samples were preserved in Queen's lysis buffer (0.01M Tris, 0.01M NaCl, 0.01M EDTA, and 1.0% n-lauroylsarcosine, pH 8.0; Seutin et al., 1991).

Nestlings were captured 12 days after hatching. Blood was collected from the brachial vein, and samples were preserved in Queen's lysis buffer, as for the adults.

I isolated genomic DNA of adult and nestling birds by using an IsoQuick nucleic acid extraction kit (ORCA Research, Bothell, WA, USA) and used polymerase chain reaction (PCR) to amplify five microsatellite loci (Table 1 in Kojima et al., 2009). I verified the presence of PCR product, and then samples were prepared for analysis on an ABI 3100 automated sequencer (Applied Biosystems). Genotypes were determined using GeneMapper™ software (Applied Biosystems). I used CERVUS, version 2.0 to calculate the exclusion probabilities for the first and second parents for each locus and to test for the presence of null alleles (Marshall et al., 1998).

I compared offspring genotypes at four loci, HrU3, HrU5, HrU6, and Pocc6, with their putative parents using CERVUS, version 2.0. I did not include HrU10 in the analysis due to the high frequency of null alleles. The four microsatellite loci had a total exclusionary power of 0.986 and 0.998 for the first and second parents, respectively. In 2005, three nestlings in two different nests out of 243 nestlings from 54 nests, showed a mismatch at all four loci with their putative mother. They were considered to be the result of intra-specific

brood parasitism. There were no cases of brood parasitism in 2006. No other cases of a genetic mismatch between offspring and social mother were recorded. I subsequently compared the offspring's paternal alleles with the alleles of the social father. The genotypes of 13 offspring were not compatible with the genotypes of their social father. Two offspring showed a mismatch at three loci and six at four loci out of four. These offspring were considered extra-pair young (EPY). Five showed a mismatch at just one locus, so in these cases I compared their genotypes at HrU10 with their social father. The results revealed that each offspring shared an allele with its putative father at HrU10. The exclusion probability of four non-mismatching loci in these chicks was  $> 0.998$  (see also Kojima et al., 2009). I concluded that the mismatches were caused by mutation and that the five offspring were within-pair young.

I used a non-parametric Mann-Whitney U-test to study the difference in ecological factors between broods with and without EPY after pooling the data across two years because of the small sample sizes. To account for duplicate observations results from seven males that bred in both 2005 and 2006, I used data from only a single year for each of these males. I used data from 2005 for four males and data from 2006 for the other three, which included one male with EPY in the brood.

## RESULTS AND DISCUSSION

The levels of extra-pair paternity were below 5% of nestlings (2.9% in 2005 and 1.9% in 2006) and below 10% of broods (7.4% in 2005 and 9.1% in 2006) in both years (Table 1), and there were no significant differences in the level of EPY and nests containing EPY between

the two years (both:  $P = 1$ , Fisher's exact test).

The observed level of EPY was the lowest among the results of the previous studies in European and North American subspecies (18–29% of nestlings and 32–52% of broods had extra-pair young; reviewed in Turner, 2006), and in studies of the colony breeders in the same subspecies in Japan (8–22% of nestlings and 12–41% of broods; Kojima et al., 2009). This figure is also small compared with the paternity of birds in general (reviewed in Griffith et al., 2002).

The low EPY level I recorded in my study might have resulted from the low population density at my study site (mean  $\pm$  SD distance between the nearest males:  $20.39 \pm 20.02$  m,  $N = 52$ ) compared with that of colony breeders in the same subspecies [the two colonies studied in Kojima et al. (2009);  $6.18 \pm 7.71$  m ( $N = 31$ ); Kitamura pers. comm.;  $t = 4.58$ ,  $P < 0.01$ ] and in other subspecies (ca. 3–5 m; reviewed in Turner, 2006). The mean distance (ca. 20 m) to the nearest male in my population, would not physically constrain extra-pair copulation in barn swallows, because they have a large home range ( $> 100$  m; reviewed in Turner, 2006). It may, however, be more difficult in a sparse population than in a dense population for males (or females) to watch for a chance to engage in extra-pair copulation with neighbours. Thus, I consider that nest density may explain the low level of paternity loss in my population.

In accordance with this, the broods with EPY had the nearest asynchronous males significantly closer than broods without EPY within my population (Fig. 1a). The relationship was probably not confounded by laying date or brood size, because these variables did not differ between the two groups (Fig. 1b and c).

These results indicate that low population density explains the low level of paternity

loss in my population. My results are consistent with the general relationship in which extra-pair paternity decreases with lowering density within species (Westneat and Sherman, 1997; Møller and Ninni, 1998; reviewed in Griffith et al., 2002). However, the current argument is only based on correlative study. Further study is needed to determine whether low paternity loss in my population can be explained by low density with experimental manipulation (e.g. Ockendon et al., 2009).

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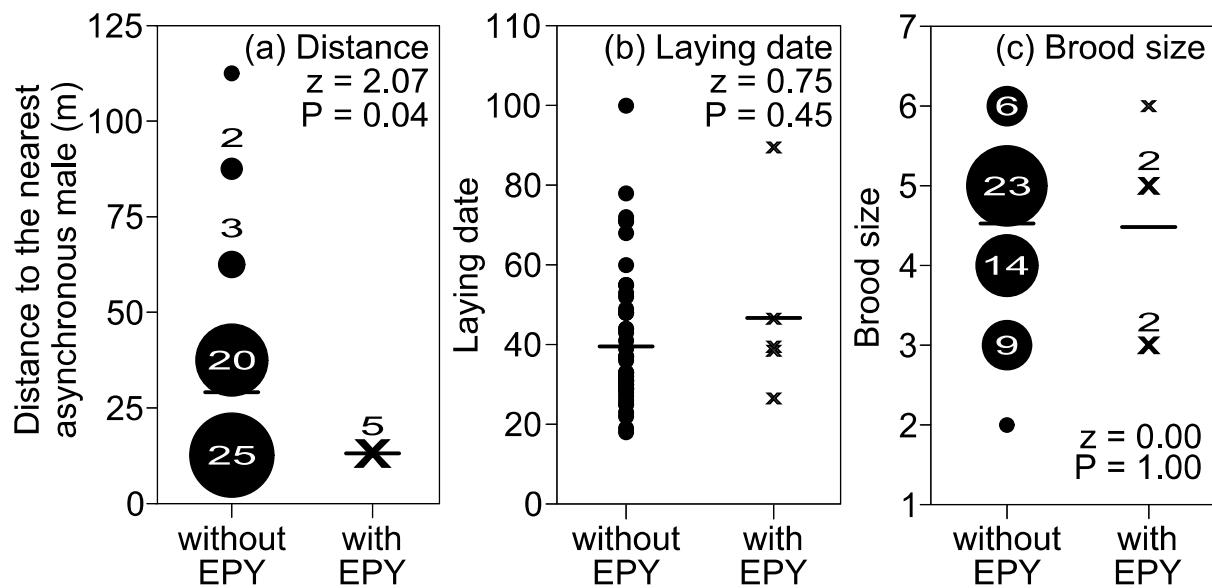
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**Table 1.** Percentage of extra-pair young in 2005 and 2006.

	Nestlings	Broods
2005	2.9% (7/243)	7.4% (4/54)
2006	1.9% (1/53)	9.1% (1/11)



**Figure 1.** Comparison of environmental variables between male barn swallows with (crosses) and without (filled circles) extra-pair young (EPY) in their broods: (a) distance to the nearest asynchronous male, (b) laying date (1 April = 1 in each year), and (c) brood size. Numbers refer to number of individuals. Horizontal lines indicate the average values in each category. Mann-Whitney U-test was used for the statistical analyses.

**CHAPTER 3**

**MATING ADVANTAGE OF MULTIPLE MALE ORNAMENTS**

**IN THE BARN SWALLOW *Hirundo rustica gutturalis*\***

\* Published in Ornithological Science, 9:141–148 (2010)

## ABSTRACT

The maintenance of multiple ornaments by animals can be explained when those multiple ornaments are sexually selected. However, there have been only a few studies of sexual selection on multiple ornaments. I investigated sexual selection on two ornaments, plumage coloration and white spots in the tail, in a population of barn swallows *Hirundo rustica gutturalis* in Japan. There was sexual dimorphism in throat coloration and in the size of the white spots in the tail. Males with a less saturated (colourful) throat and larger white spots in the tail bred earlier than others, indicating a mating advantage for these males. These trends are what would be expected if these ornaments were indeed sexually selected.

**Keywords:** ornaments; plumage coloration; tail length; throat patch; white spots in the tail

## INTRODUCTION

Sexual selection leads to ornamentation of traits that confer an advantage in mate acquisition (Andersson, 1994). There have been now many studies of sexual selection on single male ornaments (reviewed in Andersson, 1994; Hill and McGraw, 2006). However, males of many species have more than one ornament (Møller and Pomiankowski, 1993). Why do these species have multiple ornaments?

One explanation for multiple ornaments is that some ornaments, for which sexual selection has been lost, are nonetheless maintained because they are not costly (Møller and Pomiankowski, 1993). This explanation, however, cannot be applied to costly ornaments because such ornaments are easily lost through natural selection when the ornaments are no longer sexually selected (Schluter and Price, 1993). Multiple costly ornaments can be explained when the multiple ornaments are sexually selected (Møller and Pomiankowski, 1993). However, there have been only a few studies of sexual selection on multiple ornaments (e.g. the scarlet-tufted malachite sunbird *Nectarinia johnstoni*: Evans and Hatchwell, 1992a, b; the yellow-browed leaf warbler *Phylloscopus inornatus*: Marchetti, 1998; the red-collared widowbird *Euplectes ardens*: Andersson et al., 2002; the lark bunting *Calamospiza melanocorys*: Chaine and Lyon, 2008).

The barn swallow *Hirundo rustica* is a classic model species for sexual selection, because experimental manipulation has proven the male's long tail to be sexually selected (e.g. Møller, 1988; reviewed in Møller, 1994). However, it has recently been shown that long tails are also naturally selected because they provide better aerodynamic efficiency and flight manoeuvrability (e.g. Norberg, 1994; Buchanan and Evans, 2000; Cuervo and Ayala, 2005),

suggesting that sexual selection on this ornament may be less important than previously thought. Only a small proportion of the total length of the tail is sexually selected (Evans, 1998; Buchanan and Evans, 2000; Rowe et al., 2001; Bro-Jørgensen et al., 2007). Nevertheless, the barn swallow is a useful species for studying sexual selection because it has at least two other sexually selected ornaments: plumage coloration, including its red throat patch (Ninni, 2003; Safran and McGraw, 2004; Safran et al., 2005), and white spots in the tail (Kose and Møller, 1999; Kose et al., 1999). These two ornaments are shown to be costly by previous studies, some of which are experimental in nature (throat coloration: probably physiological costs: Ninni, 2003; Norris et al., 2009; Safran et al., 2010; white spots in the tail: cost of feather breakage and parasites: Kose and Møller, 1999; Kose et al., 1999), and thus seem difficult to maintain without sexual selection. However, since previous studies have focused on only one trait (i.e. the former on throat coloration and the latter on white spots in the tail), it is not known whether or not these two ornaments are sexually selected in a single population.

I studied sexual selection on two ornaments, plumage coloration and white spots in the tail, in male barn swallows *H. r. gutturalis* in Japan. For this purpose, I studied the sexual dimorphism of these ornaments and the relationship between the male ornaments and the laying date of their mates, a recognized index of mating advantage (cf. Andersson, 1994; Møller, 1994). Throat patch area and coloration are represented as plumage coloration in this study because this subspecies has a large throat patch and whitish ventral plumage (Cramps, 1988; Turner, 2006). Tail length, which is reported to be sexually selected in European populations of the barn swallows (Møller, 1994), was also included in the analysis.

## MATERIAL AND METHODS

### Study site

This study was conducted from March to August in 2005 and 2006 in a residential area of Joetsu City, Niigata Prefecture, Japan ( $37^{\circ}07' N$ ,  $138^{\circ}15' E$ ; 10 m asl.). There, barn swallows nest under the eaves of a covered sidewalk along the street and breed in a loose colony (see Tajima and Nakamura, 2003). The study site was divided into two areas. One was used to record arrival and laying date and to take measurements of the birds (males:  $n_{2005, 2006} = 110$ , 97; females:  $n_{2005, 2006} = 120$ , 89). The other was used only to take measurements (males:  $n_{2005, 2006} = 71$ , 16; females:  $n_{2005, 2006} = 69$ , 13) because I conducted a preliminary study for another purpose in this area. I included the latter area in order to provide a large sample size for sexual dimorphism, which enabled me to obtain relatively stable estimates, although qualitatively similar results were obtained when I excluded the latter one.

### Measurements

Adult swallows were captured in sweep nets while roosting at night, mainly soon after clutch completion. Each bird was provided with a standard, numbered aluminium ring and an individually recognisable combination of two or three half-sized coloured rings made from plastic rings (AC Hughes, Middlesex). The sex of an individual was determined by the presence (female) or absence (male) of an incubation patch. In 2006, adults were categorized into two classes on the basis of ringing records: (i) birds known to be  $\geq 2$  years old (i.e. birds that were known to have bred in previous seasons) and (ii) birds of unknown age. Nest ownership was subsequently determined with binoculars. At capture, I measured tarsus length,

body weight, tail length, the sizes of the white spots in the tail, and the throat patch area, and collected several throat feathers. Tail length was defined as the length of the right outermost tail feather to the nearest 0.01 mm. When analyzing laying date, I omitted from my samples all birds with broken or missing outermost tails ( $n_{2005, 2006} = 9, 12$ ). The size of the white spots in the tail was defined as the sum of the lengths of the white spots of the two outermost right tail feathers. This method was chosen to minimize the handling time of the birds.

Throat patch area was defined as the area of the swallow's red throat patch; this was measured by placing a transparent plastic sheet over the throat region, ensuring that the feathers were lying flat in their natural position, and tracing the edges of the patch on to the sheet with a marker pen (cf. Lendvai et al., 2004). I scanned the sheet and measured the area of the patch ( $\text{mm}^2$ ) using Scion Image software (Scion Corporation, Frederick, MD). Each bird's throat patch was traced twice, and the mean of the two measurements was used. The repeatability of the measurements was high (males:  $n_{2005, 2006} = 147, 112$ , repeatability $_{2005, 2006} = 0.87, 0.87$ ,  $F > 14.13$ ,  $P < 0.0001$ ; females:  $n_{2005, 2006} = 166, 100$ , repeatability $_{2005, 2006} = 0.80, 0.85$ ,  $F > 8.84$ ,  $P < 0.0001$ ; Lessells and Boag, 1987).

Once in the laboratory, I placed five throat feathers, which had been collected at capture, on a piece of white paper so that the edges of the feathers were on contact. The feather samples were scanned at 800 dpi resolution using a scanner (GT 9300 UF; Epson, Tokyo, Japan), and the images obtained were imported into Photoshop Elements 3.0 (Adobe Systems, San Jose, CA). I measured the mean red-green-blue (RGB) values for a  $30 \times 30$  pixel square near the distal end of the feather sample. The mean RGB values were converted into hue-saturation-brightness (HSB) values, using the algorithm described by Foley and van Dam

(1984). The repeatability of these variables was highly significant when birds with two feather samples were used ( $0.65 \leq$  repeatability  $\leq 0.91$ ,  $N > 30$ ,  $F > 4.69$ ,  $P < 0.001$ ; see Hasegawa et al., 2008). Details of the methodology are described elsewhere (Hasegawa et al., 2008).

Since the hue and brightness of throat coloration fades linearly with time, I corrected throat coloration using the field correction methods described by Hasegawa et al. (2008): corrected (H, S, B) = measured (H, S, B) – (0.023, 0, 0.097)  $\times$  days elapsed from the date of capture of the first bird to the date of capture of each bird. Each colour variable positively correlated with the others even after correction for seasonal colour fading (sex and year separately: H-S:  $0.33 < r < 0.81$ ,  $P < 0.0001$ ; S-B:  $0.44 < r < 0.80$ ,  $P < 0.0001$ ; B-H:  $0.81 < r < 0.94$ ,  $P < 0.0001$ ). Thus, saturation value was considered to be representative of plumage coloration, because this variable does not need to be corrected for plumage colour fading (Hasegawa et al., 2008). Positive correlations between colour variables, imply that throat plumage with a lower saturation value is generally redder (i.e. lower hue value) and darker (i.e. lower brightness value). Although Ninni (2003) and Safran and McGraw (2004) reported different relationships among colour variables (in particular, saturation was negatively correlated with other variables), it is not clear whether these differences result from population-based or method-based differences, or other reasons.

## Observation

I recorded the time of arrival of birds, which was marked in the previous years, every day and inspected nests every other day to record the laying date, which was defined as the date of laying of the first egg of the first clutch. Since I failed to capture some nesting birds during

their first clutch, I was unable to distinguish between re-nesting or the second clutch of such birds, from the first clutch of late breeding birds. Since the inclusion of these birds tended to obscure the patterns of ornaments and laying date (cf. Hill et al., 1999), my test of male ornaments and laying date was conservative. I captured about 70% of the nesting birds from both areas at the end of both seasons.

### Statistical procedures

I compared the sex differences in morphology using Welch's *t*-test (cf. Møller, 1994). To investigate the correlation among male traits, Pearson's correlation was used. I used a linear mixed-effect model (LME, 'lmer' in R package 'lme4') to examine the relationships between male ornaments and laying date. This LME framework was applied to account for the repeated usage of individuals in the both study years, by including the identity of the individuals as a random effect. The significances of the terms in the LME frameworks were based on the difference in deviance and degrees of freedom of the models with and without the predictor in question (i.e. likelihood ratio test;  $\chi^2$ -test). I statistically controlled for the effects of body condition of males and study year as additional predictors in the LME. In accordance with a previous study (Kojima et al., 2009), body condition was defined as a residual from a regression of body mass against tarsus length (General linear model: N = 288; tarsus: coefficient = 0.51,  $t = 3.77$ ,  $P < 0.001$ ; years: coefficient = -0.51,  $t = -4.24$ ,  $P < 0.0001$ ; intercept: coefficient = 12.14,  $t = 8.33$ ,  $P < 0.0001$ ).

To control for the effects of any age difference among males, in their ornaments and laying dates (Møller and de Lope, 1999), which might confound the relationship, analyses

were also conducted using a general linear model using only males known to be  $\geq 2$  years old in 2006. I did not adopt an alternative method using two male categories (i.e. males known to be  $\geq 2$  year old versus males of unknown age) as a fixed factor in a general linear model using all males captured in 2006. This was because the category ‘age-unknown’ males also included some un-ringed males that were  $\geq 2$  years old, which might confound the relationship between male ornaments and laying date. The significance of the terms in the general linear model frameworks was based on the difference in deviance and degrees of freedom of the models with and without the predictor in question (i.e. likelihood ratio test; *F*-test). For general linear model analysis using males known to be  $\geq 2$  years old, I added body condition and arrival date as additional predictors. Since pairs that reunite in subsequent years breed earlier than others (Saino et al., 2002), I excluded four males that had reunited with their mates of the previous year. However, inclusion of these males did not affect the results.

I first fitted a full model containing all explanatory variables. In the case of the LME analysis, I also included the interactions between male morphologies and study year in a full model because the relationship between each ornament and laying date might differ between study years. A final model was selected by progressively eliminating non-significant interaction terms ( $P > 0.1$ ) and then non-significant main terms. Collinearity between variables can cause problems in multiple regressions, but this is generally not considered problematic when  $|r| < 0.70$ , as in the present study (cf. Møller, 2004). In the tables, the statistics and *P* values of significant terms ( $P < 0.05$ ) are from the final model (all significant terms included), whereas statistics and *P*-values of non-significant terms are from the final model and each nonsignificant term was added separately (cf. Owens et al., 1995). When

there were influential points or outliers that substantially affected the results, I present the results both including and excluding the data points (Grafen and Hails, 2002). I also showed  $R^2$  values as improvement from null model to fitted model in the table.  $R^2$  values are not directly provided for LME. Thus, the predictive power of LME was assessed by a pseudo- $R^2$  value (in the following simply referred to as  $R^2$ ) and calculated as the  $R^2$  from a regression between predicted and observed values (Gabriel et al., 2010). The total sample size in the LME and general liner model analyses was 125 ( $n_{\text{group}} = 111$ ;  $n_{2005, 2006} = 68, 57$ ) and 22, respectively (14 males in 2005 and 13 males in 2006 were excluded because some measurements were missing). The mean values of all statistical measurements are reported with  $\pm 1$  SE. All data analyses were performed using the R (version 2.8.0) statistical package (R Development Core Team, 2008).

## RESULTS

### Sex differences and inter-correlation between ornaments

In both study years, males had less saturated throat coloration than females (Table 1). Similar results were obtained among birds known to be  $\geq 2$  years old (Table 1). Males tended to have larger throat patches (significantly larger in 2005, but not in 2006 and in birds known to be  $\geq 2$  years old; see Table 1).

Males had longer tails and larger white spots in the tail than females (Table 1). This was also the case after excluding birds of unknown age (Table 1).

Male tail length correlated positively with the size of the white spots in the tail (2005:  $r = 0.22$ ,  $N = 164$ ,  $P < 0.01$ ; 2006:  $r = 0.31$ ,  $N = 112$ ,  $P < 0.001$ ) and negatively with

the saturation value of throat plumage (i.e. males with longer tails also had colourful throats: 2005:  $r = -0.27$ ,  $N = 121$ ,  $P < 0.01$ ; 2006:  $r = -0.29$ ,  $N = 107$ ,  $P < 0.01$ ) in 2005 and 2006. Similar results were obtained for birds known to be  $\geq 2$  years old (tail length vs. the size of the white spots:  $r = 0.46$ ,  $N = 47$ ,  $P < 0.01$ ; tail length vs. saturation value:  $r = -0.28$ ,  $N = 44$ ,  $P = 0.07$ ). There were no other significant relationships among the male ornaments (all:  $P > 0.05$ ).

Male body condition correlated positively with tail length (2005:  $r = 0.22$ ,  $N = 163$ ,  $P < 0.01$ ; 2006:  $r = 0.25$ ,  $N = 111$ ,  $P < 0.01$ ) and negatively with the saturation value of throat plumage (2005:  $r = -0.24$ ,  $N = 132$ ,  $P < 0.001$ ; 2006:  $r = -0.33$ ,  $N = 106$ ,  $P < 0.001$ ). On the other hand, there were no significant relationships between male body condition and the saturation value of throat plumage ( $N = 44$ ,  $r = -0.19$ ,  $P = 0.22$ ) and other ornaments in males known to be  $\geq 2$  years old ( $N = 47$ ,  $|r| < 0.14$ ,  $P > 0.33$ ).

## Laying date

The median laying date of the first egg of the first clutch was 8 May (range = 17 April–17 June) in 2005, and 9 May (range = 21 April–13 June) in 2006. Males known to be  $\geq 2$  years old started breeding significantly earlier than males of unknown age (males known to be  $\geq 2$  years old: median date = 2 May, range = 21 April–13 May,  $N = 27$ ; age-unknown males: median date = 16 May, range = 25 April–13 June,  $N = 33$ ;  $t = 5.59$ ,  $P < 0.0001$ ). The laying dates of males known to be  $\geq 2$  years old were less variable than males of unknown age (Levene's test;  $F = 10.28$ ,  $P = 0.002$ ).

The laying date was predicted by three male ornaments: the saturation value of throat

plumage, tail length, and the size of the white spots in the tail (Table 2). Males with lower saturation values, longer tails, and larger white spots in the tail bred earlier than other males. These three ornaments also predicted the laying date in males known to be  $\geq 2$  years old (Table 3 left; Fig. 1). It should be noted that the sign of the coefficient of tail length was reversed in males known to be  $\geq 2$  years old as compared with in the LME analysis using all males. The positive relationship between tail length and the laying date was caused by an outlier (i.e. one male with a particularly long tail, whose tail was more than 2.44 SD longer than the average tail length), because the significant positive relationship between tail length and laying date disappeared when I repeated the analysis after omitting the longest-tailed male (Table 3 right).

## DISCUSSION

My results suggest that throat coloration and white spots in the tail are sexually selected in my study population of the barn swallow. First, there was sexual dimorphism in throat coloration and the size of the white spots in the tail (Table 1). Second, males with a less saturated throat and larger white spots in the tail bred earlier than others, even after controlling for the effects of male age and body condition (Table 2 & Fig. 1), indicating that such males experience a mating advantage. In fact, throat coloration was positively correlated with seasonal reproductive success even after controlling for male age (Appendix). Although I could not observe direct reproductive advantage of large white spots in the tail, males with larger white spots in the tail would have a greater reproductive advantage, because early breeding itself is advantageous for male swallows in terms of their offspring survivorship (e.g. Grüebler and

Naef-Daenzer, 2010; reviewed in Møller, 1994; Turner, 2006). These trends are what would be expected if throat coloration and white spots in the tail were indeed sexually selected.

These two ornaments, white spots in the tail and plumage coloration, have already been shown to be related to laying date in some populations and it is suggested that they are sexually selected in each population (white spots in the tail: Kose and Møller, 1999; Kose et al., 1999; plumage coloration: Safran and McGraw, 2004). However, to explain the maintenance of these two ornaments, which have been shown to be costly at least in some populations (throat coloration: Ninni, 2003; Norris et al., 2009; Safran et al., 2010; white spots in the tail: Kose and Møller, 1999; Kose et al., 1999), the two ornaments need to be sexually selected in a single population. Otherwise, the maintenance of the two ornaments is not possible or requires another mechanism to explain them (e.g. sufficient gene flow across populations with different sexual selection; reviewed in Bro-Jørgensen, 2010). This is the first study to show a relationship between laying date and the two ornaments in a single population of barn swallows. As has been shown in other species (e.g. Evans and Hatchwell, 1992a, b; Marchetti, 1998; Andersson et al., 2002; Caine and Lyon, 2008), multiple ornaments in the barn swallow can also be explained by sexual selection on the ornaments.

In the current study, male body condition was not found to be related to his mate's laying date, suggesting that body condition is less important than throat coloration and white spots in the tail at least in determining the timing of breeding of males. My results are, at first glance, inconsistent with a previous study (Kojima et al., 2009), which suggests that sexual selection favors males that are in good body condition. However, my study differs from theirs in many points (e.g. they used paternity as a response variables, measured body condition

soon after arriving, and studied a different population). Thus, I could not refer the causation of the difference between their study and ours.

Of the two remaining candidate targets of sexual selection, tail length was not found to be negatively related to laying date after controlling for the effects of male age (Table 3 & Fig. 1). This is consistent with previous studies that found no sexual selection for long tails in some other populations of barn swallows (Safran and McGraw, 2004; Neuman et al., 2007; but see Kleven et al., 2006), including a Japanese population (Kojima et al., 2009). Tail length is perhaps, therefore, less important in sexual selection in my population, at least for determining laying date than throat coloration and white spots in the tail. In addition, the other candidate, throat patch area, showed only small sexual dimorphism (Table 1) and no relationship with laying date (Table 2), suggesting that this trait is less important for breeding early.

Here, I have shown that two male ornaments, throat coloration and white spots in the tail, confer mating advantage in terms of early breeding in a population of barn swallows in Japan. Since the two ornaments are related to laying date, sexual selection can explain the maintenance of these two ornaments. However, the current study did not elucidate the mechanism of selection. Since both female mate choice and male-male competition can predict the early breeding of well-ornamented males (Wiley and Poston, 1996), further studies (e.g. manipulation experiments) are necessary to show how inter- and intra-sexual selection can maintain multiple ornaments simultaneously.

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**Table 1.** Sexual dimorphism in *Hirundo rustica gutturalis*.

				Males		Females
Red throat patch	Saturation (least saturated = 0, most saturated = 255)	2005	118.49 ± 0.39 (133)	**	121.88 ± 0.30 (152)	
	2006	109.20 ± 0.59 (107)	**	114.29 ± 0.52 (95)		
	≥ 2 years old <sup>1</sup>	107.11 ± 0.79 (44)	**	112.37 ± 0.89 (31)		
Patch area (mm <sup>2</sup> )	2005	514.88 ± 5.79 (147)	**	493.25 ± 4.80 (166)		
	2006	511.96 ± 7.65 (112)		500.04 ± 6.59 (100)		
	≥ 2 years old <sup>1</sup>	517.26 ± 11.14 (47)		499.24 ± 12.30 (32)		
Tail ornaments	Tail length (mm)	2005	94.42 ± 0.64 (164)	**	79.93 ± 0.34 (179)	
	2006	93.89 ± 0.76 (112)	**	80.23 ± 0.49 (99)		
	≥ 2 years old <sup>1</sup>	96.93 ± 1.28 (47)	**	82.42 ± 0.84 (31)		
White spots' size (mm)	2005	39.00 ± 0.41 (178)	**	29.39 ± 0.39 (184)		
	2006	37.07 ± 0.52 (112)	**	28.64 ± 0.55 (101)		
	≥ 2 years old <sup>1</sup>	38.19 ± 0.85 (47)	**	28.42 ± 0.94 (32)		

Values represented as mean ± SE; sample size in parentheses.

Differences between the sexes were tested with the *t*-test; \*\*  $P < 0.01$ .

<sup>1</sup> ≥ 2 years old included only birds known to be ≥ 2 years old from the ringing records.

**Table 2.** Results of LME analysis predicting variation in laying date using all males. Estimates of each coefficient and  $P$  values for  $\chi^2$ -test are shown. The year and its interaction with the main variables are not shown because all the relationships were non-significant ( $P > 0.1$ ).

2005+2006 (N = 125)		
Red throat patch	Saturation	† 4.47 (< 0.01)
	Patch size	0.50 (0.61)
Tail ornaments	Tail length	† -2.46 (0.03)
	White spots size	† -2.29 (0.03)
Other	Body condition	0.73 (0.47)

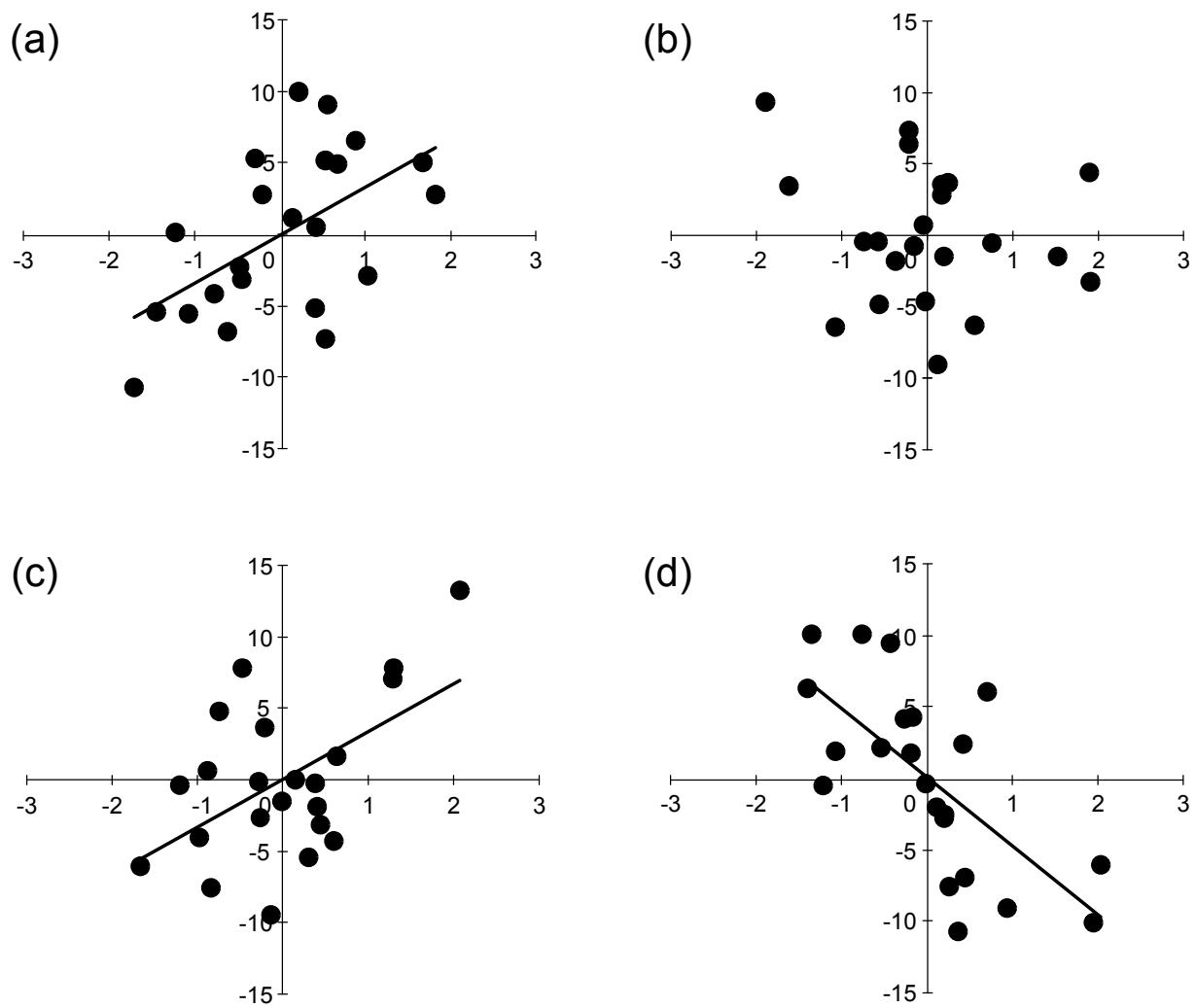
† indicates the variables retained in the final models by step-down model selection procedures.  $R^2$  of the final model was 0.83 (see Materials & Methods).

**Table 3.** Results of general linear model analyses predicting variation in laying date among males known to be  $\geq 2$  years old. Each column represents the results of the general linear model analyses: estimates of each coefficient and *P* values for *F* tests.

		All data (N = 22)	Excluding an outlier <sup>1</sup> (N = 21)
Red throat patch	Saturation	† 3.36 (0.01)	† 3.17 (0.01)
	Patch size	-0.97 (0.42)	-0.67 (0.58)
Tail ornaments	Tail length	† 3.36 (0.02)	1.83 (0.16)
	White spots size	† -4.82 (< 0.01)	† -3.67 (< 0.01)
Other	Arrival date	-0.62 (0.61)	0.20 (0.87)
	Body condition	0.63 (0.64)	0.71 (0.56)

<sup>1</sup> The analysis after omitting the longest-tailed male, whose tail length was more than 2.44 SD longer than the average tail length.

† indicates the variables retained in the final models by step-down model selection procedures.  $R^2$  values of final models were 0.53 for All data and 0.48 for Excluding an outlier.



**Figure 1.** Relationships between male ornaments (x-axis) and the date of breeding onset (y-axis), controlling for other ornaments in male barn swallows known to be  $\geq 2$  years old and excluding mate re-united birds: (a) throat coloration (saturation), (b) throat patch area, (c) tail length, and (d) the size of the white spots in the tail. Both axes show the residuals controlling for the terms retained in the final model using males known to be  $\geq 2$  years old (Table 3 left). Linear regression lines are shown.

**Appendix.** Results of LME and general linear model predicting the variation in annual reproductive success after excluding nest failure. Separate models were run for each category of males. Each column represents the results of LME (2005+2006) and general linear model ( $\geq 2$  years old): estimates of each coefficient and  $P$  values for  $\chi^2$  tests for LME and  $F$  tests for general linear model. Year and its interaction with main variables were not shown because all of the relationships were non-significant ( $P > 0.1$ ).

		2005+2006 (N = 54)	$\geq 2$ yeas old (N = 13)
Red throat patch	Saturation	<sup>†</sup> -1.14 (< 0.01)	<sup>†</sup> -1.41 (0.02)
	Patch size	0.24 (0.39)	0.68 (0.21)
Tail ornaments	Tail length	0.18 (0.54)	0.02 (0.96)
	White spots size	0.18 (0.53)	-0.03 (0.96)
Others	Body condition	-0.18 (0.58)	0.42 (0.43)

<sup>†</sup> indicates the variables retained in the final models by step-down model selection procedures.

**CHAPTER 4**

**FEMALE MATE CHOICE BASED ON TERRITORY**

**QUALITY IN BARN SWALLOWS\***

\*Submitting

## **Abstract**

Female mate choice based on territory quality is difficult to study because territories often contain many resources, which are difficult to quantify. Here, using the barn swallow (*Hirundo rustica gutturalis*), whose males defend only small territories containing old nests, I studied whether females choose social mates based on territory quality. Since the territories of this species contain few other resources, territory quality can easily be assessed in terms of the presence of old nests. I made the following four observations: (1) Male swallows displayed old nests in their territories to females. (2) The old nests used for first clutch had been less broken than the other old nests within the same territory. (3) Males with better territories, defined by the number and intactness of old nests, paired with females earlier, and hence bred earlier, than those with inferior territories. Based on these results, I can infer that female swallows choose their mates based, in part, on territory quality.

**Keywords:** *Hirundo rustica gutturalis*; mate preference; mating advantage; old nests; territory choice

## INTRODUCTION

For bird species in which males defend breeding territories, females may choose mates based on male phenotype and/or territory characteristics (e.g. Alatalo et al., 1986; Marchetti, 1998; Eckerle and Thompson, 2006; reviewed in Andersson, 1994; Jennions et al., 1997; Candolin, 2003). Among such species, there have been many studies on female mate choice for male phenotype, because this promotes intersexual selection (Andersson, 1994). Female mate choice for territory characteristics should also facilitate sexual selection by promoting male-male competition and indirect mate choice (Wiley and Poston, 1996). However, female mate choice based on territory quality is less well studied owing to the difficulty of measuring territory quality in species with all-purpose territories containing many resources that are difficult to quantify (e.g. vegetation type and density, perch site, food, and nest site; Searcy and Yasukawa, 1995).

The barn swallow (*Hirundo rustica*) is a species in which males defend only small territories, many of which contain old nests constructed in previous seasons (reviewed in Møller, 1994; Turner, 2006). Since their territories contain few other resources, territory quality can easily be assessed by the presence of old nests, as has been shown for nest site quality in certain other species [e.g. pied flycatcher (*Ficedula hypoleuca*): Slagsvold, 1986; house sparrow (*Passer domesticus*): Møller, 1988]. However, although there have been many studies on female mate choice for male phenotype in barn swallows (Safran and McGraw, 2004; Kojima et al., 2009; reviewed in Møller, 1994; Turner, 2006), no studies have directly investigated female mate choice based on territories (but see Safran, 2007 for the influence of breeding site selection on mate choice).

Møller (1990) has indirectly investigated the importance of territory quality for female mate choice from correlation analysis of settlement patterns of males in different years. The prediction is that arriving males should tend to occupy the same territories first each year if territory quality is important in determining female choice. This prediction rests on the assumption that the quality of territories remains unaltered during the period of study, as seems to be the case in his study area (Møller, 1994). Since the correlation between settlement orders in different years was low and non-significant even after excluding males that have already bred once in a study site (Møller, 1990), he argued that territory quality is unimportant for female mate choice in barn swallows.

However, choosing a male as a function of the territory he is defending has three potential advantages for female barn swallows. First, since the old nests constructed in previous breeding seasons can persist and indicate past reproductive success in the territory (Safran, 2004; Turner, 2006), it would be beneficial for females to choose a breeding site based on old nests for successful breeding. Indeed, Safran (2004, 2007) shows that female swallows prefer to breed in colonies containing many old nests, suggesting the importance of old nests as an indicator of breeding site quality. It has also been reported that females prefer to breed in old nests of high quality (e.g. fewer mites; Safran, 2006). However, since these studies focused purely on nest or breeding site choice by females, whether individual males garner a mating advantage by holding a good territory in the population or breeding site is poorly understood. Nevertheless, it is highly plausible that males holding a good territory with high quality old nests are chosen by females. Second, by using old nests, females can save time by not having to build a new nest (reviewed in Turner, 2006). Moreover, since the re-use

of old nests necessitates fewer trips to collect nesting materials, it can also reduce the risk of predation and saves energy. Third, the presence of multiple old nests in a territory, which is often seen in outdoor breeding sites (compared with indoor breeding sites such as stables), may be an additional advantage for females choosing a male territory, since multiple nests may lead to a dilution effect in predation risk (Watts, 1987; Searcy and Yasukawa, 1995). Hence, I predict that female swallows choose their mates based on the presence of old nests in male territories.

Here, I studied female mate choice based on territory quality in barn swallows nesting in an outdoor breeding site. For this purpose, I studied (i) male courtship behavior, in which important criteria of female mate choice must be shown by males (Searcy and Yasukawa, 1995); (ii) nest choice within territories as a function of old nest quality; (iii) the relationship between breeding date (pairing date and laying date) and territory quality, measured in terms of the quality and quantity of old nests in the territory.

## MATERIAL AND METHODS

### **Study site and observations**

This study was conducted in March to August 2007 in a residential area of Joetsu City, Niigata Prefecture, Japan ( $37^{\circ}07'N$ ,  $138^{\circ}15'E$ ; 10 m asl.), where the swallows nest under the eaves of a covered sidewalk along the street and breed in a loose colony (cf. Tajima and Nakamura, 2003). Each day, I recorded the time of arrival and pair formation of each marked bird (i.e. two birds behave together without courtship behavior), and inspected nests every other day to record breeding events. This allowed me to determine the laying date, which was

expressed as the date of clutch initiation. Laying date can be used as an indicator of female mate choice (cf. Andersson, 1994; Møller, 1994). Since laying date may be influenced by a number of factors other than female choice (e.g. female quality, time required for repairing old nests, weather conditions between mating and egg laying), I also used a more direct indicator of female choice, namely, pairing date, using previously marked birds.

### **Observing courtship behavior**

I observed each unmated male for at least one hour in front of its territory using a video camera-recorder (SONY CCD-TRV92). A total of 264 courtship displays performed by 42 unmated males (total 93 h) were observed from late March to early May.

### **Measuring territory quality**

In the current study, a territory was defined as the eave of one house. This is because in my study site it was rare for more than one pair of swallows to breed under each eave (Masaru Hasegawa, unpublished data). In 2007, each of all but one eave had a single breeding pair (after pair formation, one pair migrated from their original territory to an eave where another pair was already breeding, probably because of human disturbance). Each eave across the study site has a similar area (ca. 10–15 m<sup>2</sup>). Before the arrival of the birds, all the nests in the study area were scored according to one of the following five categories: 1—trace of old nest with little mud remaining; 2—small mass of mud remaining; 3—approximately half of the old nest remaining; 4—old nest with some damage; 5—old nest with little damage. Territory quality was defined in terms of the sum of the scores for all old nests in the territory. When

multiple nests were found clinging together, I classified these as a single old nest and assigned it the score of the individual nest with the highest score.

### **Capture and measurement of birds**

Adults were captured using sweep nets while roosting in the territory at night. Birds were marked with a numbered aluminum ring and an individual combination of two or three colored rings (cf. Arai et al., 2008). The sex of an individual was determined by the presence (female) or absence (male) of an incubation patch. Adults were placed into two groups—ASY (after second year) birds and age-unknown birds—based on ringing records. Nest ownership was subsequently determined using binoculars.

### **Statistical procedures**

To compare the nest scores between a used nest for first clutch and the other old nests within each territory, I used a liner mixed-effect model (LME) using the function LME (package nlme) in the R statistical package (version 2.8.0; R Development Core Team, 2008). I used a generalized linear model (GLM) using a quasi-poisson error distribution and a log link function to investigate pairing date and laying date in relation to territory quality. I did not directly compare the laying dates between the two sites (e.g. via LME) because the two study sites are adjacent, and thus, not independent with each other (e.g. immigration from nest removal site to control site). Among the breeding pairs, only one pair bred in a territory without old nests (1/84). I excluded this pair from the analysis, although their inclusion did not affect the results. This pair was excluded from the analysis so as not to confound the

quantitative difference of old nests in the territory with the effect of the presence of old nests. Since laying date is known to be affected by male age (Møller, 1994; Turner, 2006), analyses were also conducted using only ASY males to distinguish the effect of male age from that of territory quality itself. As a measure of variance accounted for by the model, we obtained pseudo- $R^2$  values (Heinzl & Mittlböck, 2003).

## RESULTS

### Courtship behavior

Figure 1 shows the sequence of courtship behavior in male barn swallows. Although some males started courting on telegraph wires, most males commenced courtship in the air. They led females to an old nest or to a potential nest site, which contained no old nest but could be used to construct a new nest, emitting typical “*wie-wie-wie*” notes (cf. Turner, 2006). Approximately one-half of the males that successfully led females to a nest were also observed to sequentially show females other nests in their territories.

### Nest choice within a territory

Each territory contained  $2.11 \pm 1.03$  old nests (mean  $\pm$  SD; range = 1–5 nests;  $N = 70$ ). When the nest scores of nests used for first clutch among pairs that had at least two old nests in their territory were compared with the scores of the other old nests within the same territory, old nests used for the first clutch were found to have significantly higher scores (LME:  $N_{\text{territory}} = 36$ ,  $N_{\text{nest}} = 91$ , Coef = 0.77,  $t = 2.54$ ,  $P = 0.01$ ).

## **Territory quality and laying date**

Mean territory quality, defined by the sum of the scores [which range from 2 to 18] for all old nests in the territory, was  $7.45 \pm 3.57$  (mean  $\pm$  SD;  $N = 67$ ). There was a negative relationship between territory quality and laying date ( $N = 67$ , Coef =  $-0.07$ ,  $F = 8.90$ , pseudo- $R^2 = 0.11$ ,  $P = 0.004$ ; open and filled circles and broad line in Fig. 2). Since there were effects of male age on laying date (ASY males:  $N = 22$ , median = 20 April, range = 12 April to 8 May; age-unknown males:  $N = 58$ , median = 2 May, range = 13 April to 8 June; Mann-Whitney  $U$ -test,  $z = 3.85$ ,  $P < 0.0001$ ) and on territory quality (ASY males:  $N = 21$ , mean  $\pm$  SE =  $9.0 \pm 0.8$ ; age-unknown males:  $N = 46$ , mean  $\pm$  SE =  $6.8 \pm 0.5$ ; Mann-Whitney  $U$ -test,  $z = 2.52$ ,  $P = 0.01$ ), age effects might have confounded the results. However, this was not the case, because the effect of territory quality remained even after excluding males whose age was not known (GLM with quasi-poisson distribution:  $N = 21$ , Coef =  $-0.12$ ,  $F = 10.74$ , pseudo- $R^2 = 0.29$ ,  $P = 0.004$ ; filled circles and narrow regression curve in Fig. 2). This relationship remained significant even when using males that did not reunite with their mate of the previous year (GLM with quasi-poisson distribution:  $N = 15$ , Coef =  $-0.14$ ,  $F = 10.31$ , pseudo- $R^2 = 0.36$ ,  $P < 0.01$ ).

## **Territory quality and pairing date**

I also found a negative relationship between pairing date and territory quality in ASY birds (GLM with quasi-poisson distribution:  $N = 18$ , Coef =  $-0.18$ ,  $F = 9.29$ , pseudo- $R^2 = 0.30$ ,  $P = 0.008$ ; Fig. 3). This relationship remained significant even when using males that did not reunite with their mate of the previous year (GLM with quasi-poisson distribution:  $N = 13$ , Coef =  $-0.16$ ,  $F = 6.81$ , pseudo- $R^2 = 0.28$ ,  $P = 0.02$ ). There was a similar relationship between

pairing date and territory quality after controlling for arrival date (GLM with quasi-poisson distribution:  $N = 18$ ; territory quality: Coef =  $-0.15$ ,  $F = 5.52$ ,  $P = 0.03$ ; arrival date: Coef =  $0.06$ ,  $F = 4.03$ ,  $P = 0.06$ ;  $F = 7.11$ , pseudo- $R^2 = 0.40$ ,  $P < 0.01$ ). Among the 18 males for which the pairing date was known, four males changed their territory before their mating status was confirmed (i.e. unmated or mated). Hence, it is possible that some of these birds changed breeding territory after pair formation. However, excluding these four birds did not alter the relationship between pairing date and territory quality (GLM with quasi-poisson distribution:  $N = 14$ , Coef =  $-0.18$ ,  $F = 13.62$ , pseudo- $R^2 = 0.46$ ,  $P = 0.003$ ). This was also the case when controlling for the effect of arrival date (GLM with quasi-poisson distribution;  $N = 14$ ; territory quality: Coef =  $-0.15$ ,  $F = 8.34$ ,  $P = 0.01$ ; arrival date: Coef =  $0.04$ ,  $F = 2.01$ ,  $P = 0.18$ ;  $F = 7.95$ , pseudo- $R^2 = 0.50$ ,  $P < 0.01$ ).

## DISCUSSION

In this study, I showed that males displayed old nests to females during their courtship behavior, suggesting the importance of old nests in attracting potential mates (Figure 1). Within territories, old nests of higher quality were used as breeding nests. Males having many high-quality old nests in their territory bred earlier than others (Figs 2 & 3). These results are consistent with the idea that female barn swallows chose their social mates, in part, based on territory quality. Since I used completeness of old nests as the criterion of territory quality, the early laying date in good territories may be attributable to the fact that pairs in these territory spend less time repairing old nests before laying a clutch than those in less good territories (e.g. Turner, 2006; Safran, 2006). However, this explanation could not account for the early

mating date of males with good territories (Fig. 3). Thus, I conclude that female swallows choose their mates based, in part, on territory quality. This conclusion does not change even after controlling for male ornaments, such as tail length (Hasegawa et al., in preparation).

My finding is inconsistent with the argument that territory quality is unimportant in barn swallows (Møller, 1990, 1994). Møller's argument is based on the observation that the correlation between settlement orders in different years was low and non-significant, which should be positively correlated if territory quality is important. However, his argument rests on some assumptions (Møller, 1994) and is not a necessary condition for female mate choice based on territory quality. To the best of my knowledge, the current study is the first to show the results consistent with female mate choice based on territory quality in barn swallows. This study reinforces the previous finding that females choose their breeding site (colony choice: Safran, 2004, 2007; breeding nests: Safran, 2006), and further demonstrates that females use similar criteria when choosing their mates.

Even females of a species that has few resources within its territories might choose their mates based on territory quality. Female mate choice based on territory quality has also been shown in certain other species (e.g. pied flycatcher: Slagsvold, 1986; house sparrow: Møller, 1988). Thus, territories containing few resources should not simply be assumed to be unimportant, which has been the case in some studies (e.g. Møller, 1994; Carty et al., 1999; Friel and Klump, 1999). Rather, for the ease of measuring territory quality, these species should be used as model species to study female mate choice based on territory quality, which is difficult to accomplish in species with territories containing many resources.

Here, I showed female mate choice based on territory quality in the barn swallow, which is a model species for sexual selection (Møller, 1994). Sexual selection studies in this species have focused exclusively on female mate choice for male phenotype (reviewed in Møller, 1994). However, female mate choice based on territory quality could have an influence on sexual selection by promoting male-male competition and indirect mate choice for male phenotype (Wiley and Poston, 1996), and by suppressing direct female mate choice for male phenotype in some environments (reviewed in Candolin, 2003). To complete the picture of sexual selection based on male phenotype, not only male ornamentation itself but also the territory, should be studied.

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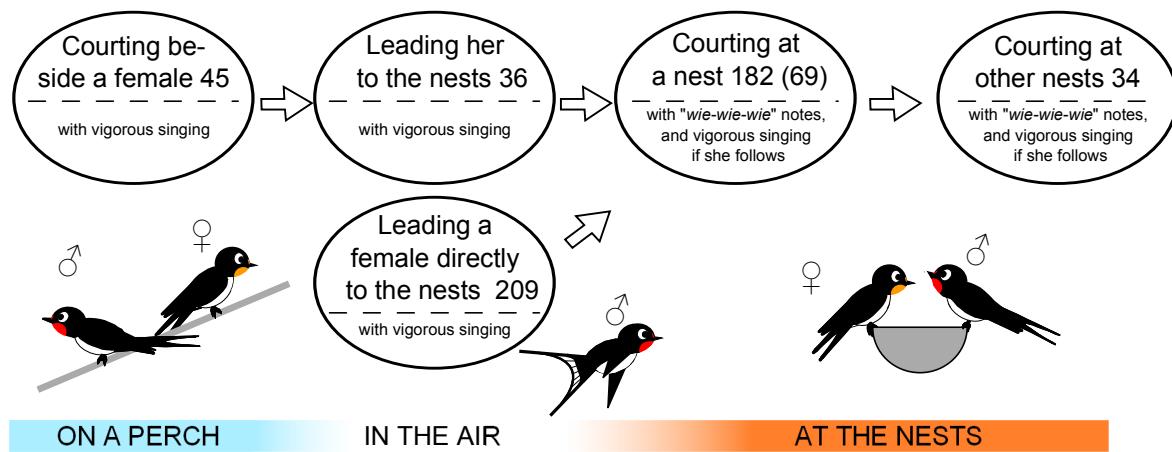
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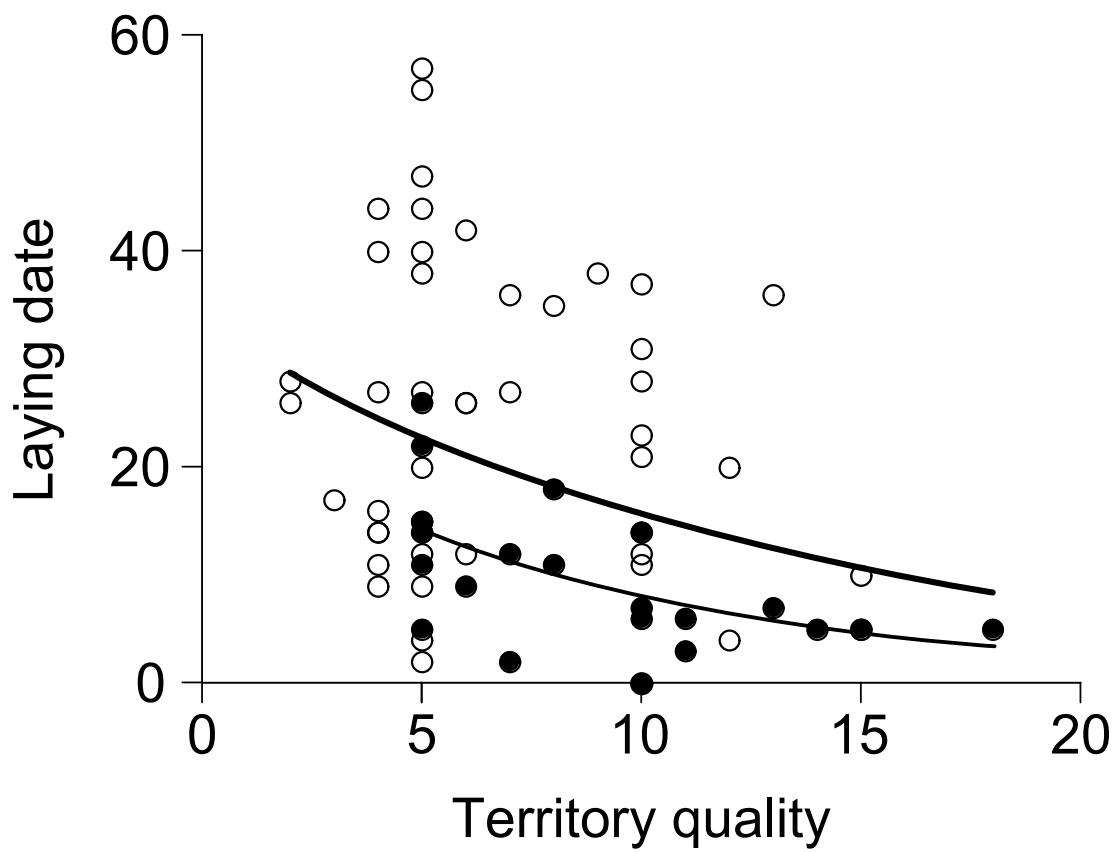
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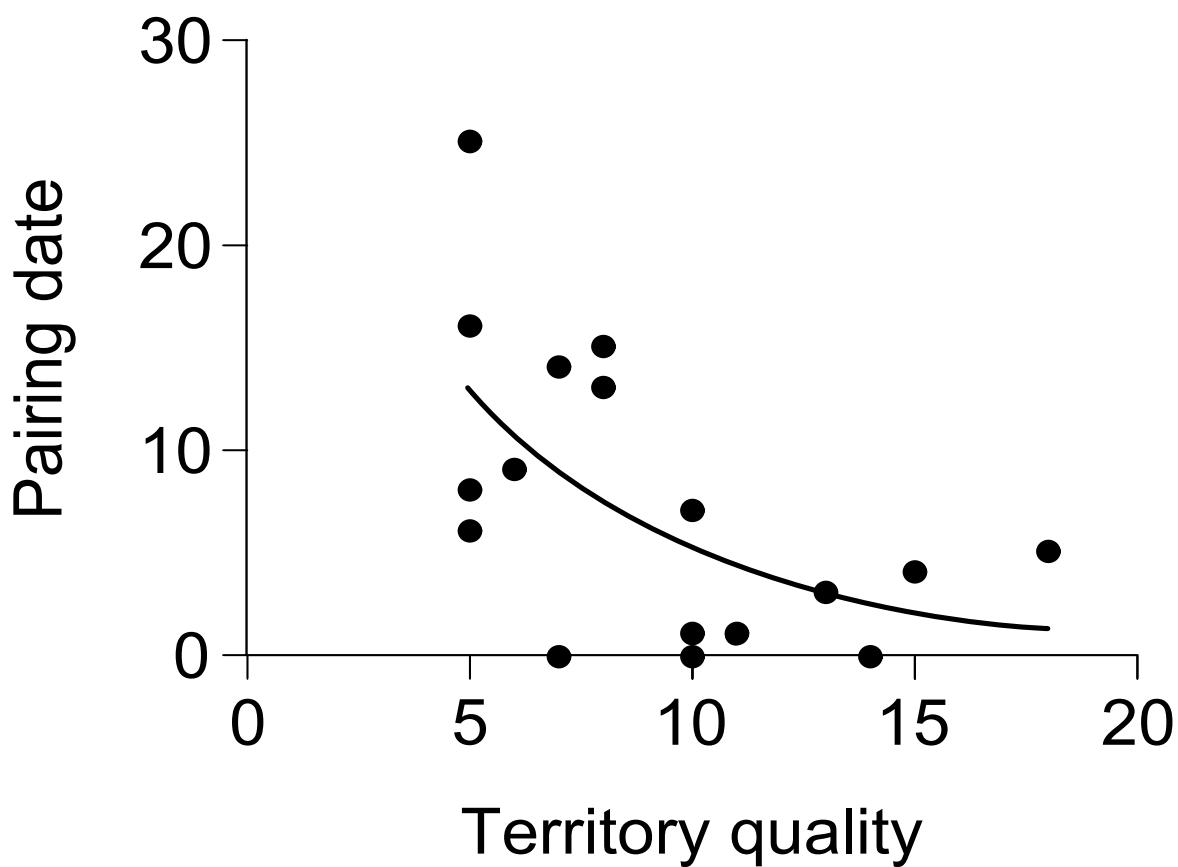
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**Figure 1.** Courtship sequence in the barn swallow. The value in each cell indicates the number of behaviors observed (The value in the parentheses indicates the number of instances in which males successfully attracted females to their nests).



**Figure 2.** Relationship between male territory quality and the laying date (12 April = 0) of his mate. Filled and open circles indicate ASY and age-unknown males, respectively. Regression curves are shown (broad line: ASY and age-unknown males; narrow line: ASY males only).



**Figure 3.** Relationship between male territory quality and pairing date (31 March = 0) in ASY males. A regression curve is shown.

## CHAPTER 5

### **Intra- and intersexual selection for multiple ornaments in barn swallows *Hirundo rustica gutturalis*\***

\*Preparing

## ABSTRACT

Multiple ornaments can be explained by the multiple receiver hypothesis, in which intrasexual selection favors an ornament and intersexual selection favors another. However, only a handful of studies support this hypothesis, probably because of the difficulty in decoupling the two sexual selection mechanisms. In this study, I tested the multiple receiver hypothesis by using the barn swallow *Hirundo rustica gutturalis*, which defends small territories around their breeding nests. Territory quality, the target of intrasexual selection, increased with decreasing saturation of throat color in male territory owners, suggesting that less saturated throat coloration (i.e., more colorful) was favored by intrasexual selection for good territory. In contrast, the size of the white spots in the tail, the other sexually selected trait, was not associated with territory quality. Instead, this ornament was associated with laying date after controlling for territory quality, suggesting that this ornament was favored by intersexual selection. These results are consistent with the multiple receiver hypothesis in barn swallows.

**Keywords:** multiple receivers; color patch, sexual selection; tail length; territory quality

## INTRODUCTION

Why animal species sometimes develop multiple sexually selected ornaments instead of concentrating on a single ornament? Several adaptive explanations have been proposed for this question (e.g. obsolete signal hypothesis: Pomiankowski and Iwasa, 1993; multiple message hypothesis: Johnstone, 1996; van Doorn and Weissing, 2006; dynamic female choice: Bro-Jorgensen, 2010). Although most of these studies focused only on intersexual selection through female mate choice, intrasexual selection (e.g. through male-male competition for territories) as well as intersexual selection can also be responsible for multiple ornaments (Andersson et al., 2002). If an ornament is favored by intrasexual selection and another ornament is favored by intersexual selection, both male ornaments are adaptively maintained (“multiple receiver hypothesis”; Andersson et al., 2002).

The coexistence of distinctly different signals used in mate choice and male aggressive competition has been demonstrated or strongly indicated in a number of animals (Andersson, 1994; Andersson et al., 2002). However, only a handful of studies have explicitly discussed multiple ornaments and have supported the multiple receiver hypothesis (Marchetti, 1998; Andersson et al., 2002; Loyau et al., 2005; Tarof et al., 2005; Karbian et al., 2009). The scarcity of research on this topic might be due to the difficulty in decoupling intersexual selection from intrasexual selection, because the two selections predict the same results in many cases. For example, well-ornamented males can acquire females quickly when male ornamentation is the target of female mate choice (i.e. intersexual selection), as well as when male ornamentation is the target of male-male competition for territories that are preferred by females (i.e. intrasexual selection; Wiley and Poston, 1996).

Decoupling intersexual selection from intrasexual selection can be achieved by controlling for the target of intrasexual selection (e.g. territory quality) statistically or experimentally. If a male ornament is favored even after controlling for the target of intrasexual selection, it would be intersexually selected. However, only a few studies have controlled for the target of intersexual selection (e.g. Bart and Earnst, 1999), especially in the context of multiple ornaments. Intrasexual selection should be studied in species in which the target of intrasexual selection can be easily measured.

In the previous study, the two male ornaments, throat coloration and white spots in the tail, were found to be sexually selected in a single population of the barn swallow *Hirundo rustica* in Japan (Hasegawa et al., 2010). Although most of the previous studies on sexual selection in this species have focused solely on intersexual selection, female swallows also choose their mates according to territory quality (Hasegawa et al., in prep.), suggesting the potential confounding effect of male-male competition for territory quality (i.e. intrasexual selection; cf. Wiley and Poston, 1996). However, since territory quality can be easily measured in my population (Hasegawa et al., in prep.), it should be possible to decouple intersexual selection from intrasexual selection for territory, if it exists. Intrasexual selection for territory is plausible, because good territory is limited in the population (Hasegawa et al., in prep.; this study) and because males that can secure high-quality territory are able to breed in good environments and have a mating advantage owing to their good territory (Wiley and Poston, 1996). Thus, I predict that the barn swallow is the good model to study the multiple receiver hypothesis in the natural environment by decoupling intersexual selection from intrasexual selection for territory.

In this study, I studied whether the maintenance of the two sexually selected ornaments, throat coloration and white spots in the tail, in the Asian barn swallow *Hirundo rustica gutturalis* can be explained by the multiple receiver hypothesis. First, I examined the relationship between territory quality and male ornaments (i.e. intrasexual selection). If well-ornamented males are successful in competition for good territories, there should be a positive relationship between territory quality and male ornament expression (e.g. Wolfenburger, 1999). Second, I investigated the relationship between breeding onset (the timing of pairing and that of laying) and male ornaments with and without statistically controlling for territory quality. If well-ornamented males are chosen by females (i.e. intersexual selection), well-ornamented males would breed earlier than others even after controlling for territory quality. If the two ornaments are maintained according to the multiple receiver hypothesis, different male ornaments would be related to territory quality and laying date.

## MATERIAL AND METHODS

### Study site and observations

This study was conducted from March to August 2007 in a residential area of Joetsu City, Niigata Prefecture, Japan ( $37^{\circ}07'N$ ,  $138^{\circ}15'E$ ; 10 m asl.). In this area, the swallows nest under the eaves of a covered sidewalk along the street and breed in a loose colony (cf. Tajima and Nakamura, 2003). The time of arrival and pair formation of each marked bird were recorded daily and the nests were inspected every other day to record breeding events. This allowed the determination of the laying date, which was the date that the

first egg of the first clutch was laid. Laying date can be used as an indicator of female mate choice (Andersson, 1994; Møller, 1994).

### **Measuring territory quality**

In the current study, a territory was defined as the eave of one house. This is because in my study site it was rare for more than one pair of swallows to breed under each eave (Masaru Hasegawa, unpublished data). Each eave across the study site has a similar area (ca. 10–15 m<sup>2</sup>). Before the arrival of the birds, all the nests in the study area were scored according to one of the following five categories: 1, trace of old nest with little mud remaining; 2, small mass of mud remaining; 3, approximately half of the old nest remaining; 4, old nest with some damage; and 5, old nest with little damage. Territory quality was defined in terms of the sum of the scores for all old nests in the territory. The detailed method is described elsewhere (Chapter 4).

### **Capture and measurement of birds**

Adult birds were captured while roosting at night, with the use of sweep nets. The birds were fitted with a numbered aluminum ring and an individual combination of two or three colored rings (cf. Arai et al., 2008). The sex of the bird was determined by the presence (female) or absence (male) of an incubation patch. The adults were categorized into two groups, ASY (after second year) birds and age-unknown birds, based on ringing records. Nest ownership was subsequently determined using binoculars.

At capture, I measured tail length, the sizes of the white spots in the tail, and throat

patch height, and collected some throat feathers. Tail length was defined as the right outermost tail feather to the nearest 0.01 mm. The size of the white spots in the tail was defined as the sum of the length of the white spots of the two outermost tail feathers on the right side (Hasegawa et al., 2010). I also recorded male body weight, although I failed to weigh some males for mechanical trouble. Although the previous study used body condition, defined as a residual from regression of the body weight to tarsus length (Kojima et al., 2008; Hasegawa et al., 2010), I used body weight itself as a variable because of no significant relationship between body weight and tarsus length in the current sample ( $N = 25$ ,  $r = 0.12$ ,  $P = 0.56$ ).

Throat patch height was defined as the height of the swallow's red throat patch. I used throat patch height rather than throat patch area in the current study, because of the easiness of measurement. I placed a transparent plastic sheet on the throat region, ensuring that the feathers lay flat in their natural position, and traced the size of the patch on the sheet with a marker pen (cf. Lendvai et al., 2004). I scanned the sheet and measured the length of the patch (mm) using Scion Image software (Scion Corporation, Frederick, MD). Each bird's throat patch was traced twice and the mean of the two measurements was used. Detailed method was described elsewhere (Hasegawa et al., 2010).

Once in the laboratory, I piled five feathers on a piece of white paper so that the perimeters of the feathers coincided. The feather samples were scanned at 800 dpi resolution using an EPSON GT 9300 UF scanner, and the images obtained were imported into the Adobe Photoshop Elements 3.0 program (Adobe Systems, USA). I measured mean RGB-values in a square of 30 pixels near the distal end of the feather sample. The

mean RGB-values were converted to hue-saturation-brightness (HSB)-values by the algorithm described in Foley and van Dam (1984). Among these three color variables, saturation value is taken as the representative of plumage coloration, because this variable needs not to be corrected for plumage color fading (Hasegawa et al., 2008). Detailed method was described elsewhere (Hasegawa et al., 2008).

### Statistical procedures

To know the general trends in the population, timing of laying was defined as the number of days from the first laying date in the population to the laying date of the mate of the focal male, and its relationship with male ornaments was analyzed (cf. Møller, 1994). I used a generalized linear model (GLM) using a quasi-poisson error distribution and a log link function to investigate pairing date and laying date in relation to male ornaments, with and without statistically controlling for territory quality. Timing of pairing was similarly defined and analyzed. As a measure of variance accounted for by the model, we obtained pseudo- $R^2$  values (Heinzel & Mittlböck, 2003).

Although observing the trends in the population as a whole is a useful approach, such trends may obscure the fine detail and reality of individual male-male competition and female mate choice (cf Buchanan and Catchpole, 1998). Since I can never be sure how many territories and which ones are visited by males before male territory choice, I focused on the two males holding the two nearest neighbor territories, which would be visited by these males. I was able to identify 10 such matched males, and compared their ornamentation in relation to the difference of territory quality. If well-ornamented males have higher ability to hold high-quality

territories, males with better territories would have better expression of ornaments than males with worse territories in each dyad. Similar analyses were done for female mate choice using laying date. If well-ornamented males have higher ability to access to females, males that bred earlier would have better expression of ornaments than males that bred latter in each dyad. For these analyses, I used paired *t*-test or Wilcoxon signed-rank test, as appropriate.

I used simple Pearson's correlation coefficient to study correlation between variables. I applied the Bonferroni correction to reduce the number of cases with significance arising by chance because of multiple comparisons. I did not use multivariate analysis (e.g. multiple regression), because of small sample sizes and correlations among variables (e.g. positive correlation between tail length and the size of white spots; Appendix). All data analyses were performed using the R (version 2.8.0) statistical package (R Development Core Team, 2008).

## RESULTS

### Descriptive statistics

Table 1 shows the descriptive statistics of the current sample. ASY males were heavier and had longer tails, larger white spots in the tail, larger throat patch, and less saturated throat coloration than age-unknown males, although the difference in throat coloration was marginally significant (Table 1). In addition, ASY males occupied better territories and bred earlier than age-unknown males.

### Territory change of ASY males

In ASY males, about 60 % of males settled on the same territories they occupied in the

previous year (10/17). Among the remaining seven males, which moved into a new territory, all but one male moved without their previous territories being occupied by other males. The previous territories of these six males were significantly lower than that of males returning to their previous territory (Fig. 1, left and right). By moving into a new territory, the six males improved their territory quality (Fig. 1, left and middle). However, their new territories were still lower quality than that of males returning to their previous territory (Fig. 1, middle and left). There were no significant differences between moved males and unmoved males in four ornaments ( $N_{\text{moved, stay}} = 6, 10$ ,  $|t| < 0.85$ ,  $P > 0.41$ ), body weight ( $N_{\text{moved, stay}} = 3, 8$ ,  $t = -0.91$ ,  $P = 0.40$ ), and arrival date ( $N_{\text{moved, stay}} = 6, 8$ ,  $t = -1.07$ ,  $P = 0.32$ ).

### Trends of the territory quality

Territory quality increased with decreasing throat saturation of male territory owner (i.e. good territories were occupied by colorful males; Fig. 2; all circles and broad regression line). This relationship was marginally significant after controlling for the number of ornaments focused (i.e. four) by Bonferroni correction ( $\alpha = 0.05/4 = 0.0125$ ). No significant correlations were observed between territory quality and the three other male ornaments ( $N = 31$ ,  $|r| < 0.11$ ,  $P > 0.51$ ), and male body weight ( $N = 25$ ,  $r = 0.14$ ,  $P = 0.50$ ).

In ASY males, there was a weak, nonsignificant, correlation between territory quality and the throat saturation of male territory owner (Fig. 2; filled circles and hatched regression line). Territory quality was not correlated with the other three male ornaments ( $N = 17$ ,  $|r| < 0.28$ ,  $P > 0.28$ ), male body weight ( $N = 12$ ,  $r = 0.22$ ,  $P = 0.48$ ), and male arrival date in ASY males ( $N = 15$ ,  $r = -0.40$ ,  $P = 0.15$ ).

### **Matched pair analysis**

Within the two nearest neighbors, males with better territories had less saturated throat coloration than males with worse territories (Fig. 3), even after Bonferroni correction ( $\alpha = 0.0125$ ). Use of a one-tailed test was justified by the trends observed in the population (Fig. 2). There were no significant differences between dyads in the other three male ornaments (10 pairs, paired *t*-test,  $|t| < 0.49$ ,  $P > 0.64$ , two-tailed) and male body weight (6 pairs, paired-*t* test,  $t = 0.26$ ,  $P = 0.81$ , two-tailed).

Since there was a significant age-difference between the two male groups (10 pairs, Wilcoxon signed-rank test,  $V = 0$ ,  $P = 0.036$ , one-tailed), male-age would be confounded with the difference in male throat coloration between the two groups. However, this might not be the case, because there was still a significant difference in throat coloration between the two groups even when using males with matched age-class (i.e. the two neighbors were both ASY males or both age-unknown males; Fig. 3; black and gray lines). This difference remained significant after Bonferroni correction ( $\alpha = 0.0125$ ). There were no differences in the other three male ornaments (6 pairs, paired *t*-test,  $|t| < 1.43$ ,  $P > 0.21$ , two-tailed) and male body weight (Body weight, 4 pairs,  $t = -0.08$ ,  $P = 0.94$ , two-tailed), although sample sizes were small.

### **Trends of the timing of laying**

In the analysis of all males, males with better territories bred earlier than others (Table 2). In addition, males with longer tails and larger white spots in the tail also bred earlier than others whether or not controlling for territory quality (Table 2). The timing of laying was not significantly related to male throat coloration and throat patch height (Table 2), and male body

weight whether or not controlling for territory quality (without:  $N = 25$ , Coef. =  $-0.22$ ,  $F = 2.54$ , pseudo- $R^2 = 0.06$ ,  $P = 0.12$ ; with:  $N = 25$ , Coef.<sub>weight</sub> =  $-0.21$ ,  $F = 2.23$ ,  $P = 0.15$ ; Coef.<sub>territory</sub> =  $-0.24$ ,  $F = 3.17$ ,  $P = 0.09$ ;  $F = 2.95$ , pseudo- $R^2 = 0.15$ ,  $P = 0.07$ ).

In the analysis of ASY males, males with better territories bred earlier than others (Table 2). Males with less saturated throat tended to breed earlier than others without controlling for territory quality (Table 2). No other male ornaments were correlated with the timing of laying without controlling for territory quality (Table 2). When controlling for territory quality, white spots in the tail, but not other three male ornaments, were marginally correlated with this index (Table 2). Male body weight was not related to the timing of laying in ASY males with and without controlling for territory quality (without:  $N = 12$ , Coef. =  $-0.16$ ,  $F = 0.61$ , pseudo- $R^2 = -0.04$ ,  $P = 0.45$ ; with:  $N = 12$ , Coef.<sub>weight</sub> =  $-0.06$ ,  $F = 0.16$ ,  $P = 0.70$ ; Coef.<sub>territory</sub> =  $-0.47$ ,  $F = 10.34$ ,  $P = 0.01$ ;  $F = 5.80$ , pseudo- $R^2 = 0.43$ ,  $P = 0.02$ ).

### **Matched pair analysis**

The former breeders within the two nearest neighbors had better territories than the latter breeders (Table 3). The former breeders had also less saturated throat than the latter breeders (Table 3), although the difference was not significant after Bonferroni correction ( $\alpha = 0.0125$ ). In addition, the former breeders tended to have longer tails and larger white spots in the tail than the latter breeders (Table 3). However, these differences of male ornaments should be confounded by that of territory quality. After controlling for territory quality by using residuals from regression of each male ornament to territory quality, residual white spots in

the tail and residual tail length, but not other two residual ornaments, were significantly differed between dyads, although neither variable was significant after Bonferroni correction (Table 3). There was no significant difference in male body weight with and without controlling for territory quality between dyads (without: 6 pairs,  $t = 0.50$ ,  $P = 0.64$ , two tailed; with: 6 pairs,  $t = 0.26$ ,  $P = 0.81$ , two-tailed).

There was a significant age-difference between dyads (10 pairs,  $V = 10$ ,  $P = 0.036$ , one-tailed), which might be confounded with the differences in male ornaments. In males with matched age-class, territory quality did not differ between dyads (Table 3). The former breeders tended to have larger white spots than the later breeders with and without controlling for territory quality than the latter breeders (Table 3). No other male ornaments differed between dyads with and without controlling for territory quality (Table 3). There was also no significant difference in male body weight with and without controlling for territory quality (without: 4 pairs, paired  $t$ -test,  $t = 0.14$ ,  $P = 0.90$ ; with: 4 pairs,  $t = 0.07$ ,  $P = 0.95$ ), although sample sizes were small.

### The timing of pairing

The timing of pairing was significantly negatively correlated with territory quality ( $N = 15$ ,  $r = -0.69$ ,  $P < 0.01$ ). It was not significantly correlated with any of male ornaments ( $N = 15$ ,  $|r| < 0.36$ ,  $P > 0.18$ ), even after controlling for territory quality ( $N = 15$ ,  $|\text{partial } r| < 0.41$ ,  $P > 0.12$ ). There was a positive relationship between arrival date and the timing of laying ( $N = 13$ ,  $r = 0.67$ ,  $P < 0.01$ ). Thus, this relationship may be confounded with the relationship between the timing of pairing and four male ornaments, male body weight and territory quality, if arrival date was correlated with these traits. However, this might not be the case,

because arrival date was not significantly correlated with four male ornaments ( $N = 15$ ,  $|r| < 0.23$ ,  $P > 0.41$ ), male body weight ( $N = 10$ ,  $r = 26$ ,  $P = 0.46$ ), and territory quality ( $N = 15$ ,  $r = -0.40$ ,  $P = 0.14$ ). Unfortunately, I could not compare male ornaments and territory quality in relation to the order of pairing between the two nearest neighbor males for small sample size ( $N = 3$ ).

## DISCUSSION

The current study supports the multiple receiver hypothesis as an explanation for the two male ornaments, throat coloration and white spots in the tail, in male barn swallows. First, throat coloration would be sexually selected through male-male competition for high-quality territory (i.e. intrasexual selection). This was based on the observation that colorful males had greater ability to hold high-quality territory than others (Figs. 2 and 3). High-quality territory provides males with a reproductive advantage, as in the case of females (Hasegawa et al., in prep.), as well as a mating advantage due to their good territory (Tables 2 and 3; cf. Wiley and Poston, 1996; see also Hasegawa et al., in prep.). The reason why only certain (i.e. colorful) males hold good territory may be the limitation of high-quality territory in the population, because males that abandon their previous territory could not hold a territory with quality as high as that of nonmoving males (Fig. 1), and ASY males hold higher-quality territories than males (Table 1; Hasegawa et al., in prep.) that arrive later in the season (Masaru Hasegawa unpublished data; Turner, 2006). The alternative explanation that not territory quality but colorful males are directly chosen by females might not be the case, because the two indexes of female choice, laying date and pairing date, are always tightly

correlated with territory quality than with male throat coloration (Table 2). These results suggest that intrasexual selection for good territory favors males with colorful throat in this species. This inference is reinforced by a previous study showing high testosterone levels in colorful males (Safran et al., 2008), which suggests the high competitive ability of these males (e.g. Hegner and Wingfield, 1987).

Second, white spots in the tail, the other sexually selected ornament in my population (Hasegawa et al., 2010), would be favored not by intrasexual selection but by intersexual selection. This ornament was not associated with territory quality but was associated with laying date even after controlling for territory quality (Table 2). Because white spots in the tail was significantly correlated with the timing of laying, but not with that of pairing, this ornament would be used in differential reproductive investment after pairing (Sheldon, 2000) as well as female mate choice. This view is consistent with the results of previous experimental studies in which the size of the white spots in the tail is manipulated (Kose and Møller, 1999; Kose et al., 1999).

Only a few studies have supported the multiple receiver hypothesis (Evans et al., 1992a, b; Marchetti, 1998; Andersson et al., 2002; Loyau et al., 2005; Tarof et al., 2005; Karubian et al., 2009), probably because of the difficulty in decoupling the two sexual selection mechanisms. By controlling for territory quality, the target of intrasexual selection, I was able to demonstrate that intersexual selection and intrasexual selection favor different male ornaments in barn swallows. This is the first study to show the importance of intrasexual selection for male ornaments in this model species of sexual selection (Møller, 1994). Quantifying territory quality would help determine the function of male ornaments (i.e. inter-

and intrasexual selection) in territorial species (see also Bart and Earnst, 1999).

Our results are consistent with the multiple receiver hypothesis in barn swallows. The multiple receiver hypothesis should be studied by decoupling intersexual selection and intrasexual selection by focusing the target of intrasexual selection. However, the reason and mechanism behind the use of the two ornaments in intra- and intersexual selection were not addressed by the current study. Future studies should focus on these aspects.

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**Table 1.** Descriptive statistics (Mean  $\pm$ SD) of the variables used in the analysis.

Variables	age-unknown males (N = 14)	ASY males (N = 17)	t	P
Body weight <sup>1</sup>	17.36 $\pm$ 0.75	18.23 $\pm$ 0.84	2.72	0.01
Throat patch height	26.46 $\pm$ 3.93	30.77 $\pm$ 2.59	3.53	<0.01
Throat coloration	104.83 $\pm$ 3.97	100.14 $\pm$ 8.59	-2.01	0.056
Tail length	92.85 $\pm$ 7.76	101.10 $\pm$ 5.64	3.32	<0.01
Size of white spots in the tail	35.48 $\pm$ 8.40	41.07 $\pm$ 5.29	2.16	0.04
Territory quality	7.00 $\pm$ 3.42	9.76 $\pm$ 3.73	2.15	0.04
Timing of laying <sup>2</sup>	21.07 $\pm$ 13.39	9.41 $\pm$ 7.20	-2.93	<0.01
Timing of pairing <sup>3</sup>	—	6.67 $\pm$ 7.52	—	—

<sup>1</sup>Sample sizes are reduced to 13 in age-unknown males and 12 in ASY males.

<sup>2</sup>This was defined as the number of days from the first laying date in the population to each laying date (range: 2-47 for age-unknown males, 0-26 for ASY males).

<sup>3</sup>This was defined as the number of days from the first pairing date in the population to each pairing date (range: 0-25 for ASY males). Sample size is reduced to 15 in ASY males. The timing of pairing of age-unknown (i.e. unbanded) males could not be quantified.

**Table 2.** Relationship between male ornaments and laying date with and without controlling for territory quality in all males and in ASY males.

	Simple GLM		Multiple GLM		
	Coefficient	pseudo- $R^2$	Coef ornament	Coef territory	pseudo- $R^2$
<b>All males (N = 31)</b>					
Throat patch height	-0.18 (0.20)	0.02	-0.24 (0.10)	0.34 (0.03)	0.15
Throat coloration	0.23 (0.15)	0.04	-0.15 (0.42)	-0.25 (0.14)	0.08
Tail length	<b>-0.45 (&lt;0.01)</b>	0.26	-0.48 ( <b>&lt;0.01</b> )	-0.32 ( <b>0.01</b> )	0.39
Size of the white spots in the tail	-0.35 (0.02)	0.16	-0.43 ( <b>&lt;0.01</b> )	-0.40 ( <b>&lt;0.01</b> )	0.34
Territory quality	-0.29 (0.06)	0.09	—	—	—
<b>ASY males (N = 17)</b>					
Throat patch height	0.11 (0.59)	-0.05	0.23 (0.13)	-0.57 ( <b>&lt;0.01</b> )	0.41
Throat coloration	0.25 (0.21)	0.04	0.11 (0.56)	-0.50 (0.02)	0.34
Tail length	-0.05 (0.82)	-0.06	0.01 (0.97)	-0.53 ( <b>0.01</b> )	0.33
Size of the white spots in the tail	-0.28 (0.17)	0.07	-0.33 (0.049)	-0.55 ( <b>&lt;0.01</b> )	0.48
Territory quality	<b>-0.53 (0.01)</b>	0.36	—	—	—

GLM with quasipoisson distribution was used.

*P*-values are shown in the parentheses.

Bold *P*-values indicate significance after adjustment of multiple comparison (territory quality,  $\alpha = 0.05$ ; four male ornaments,  $\alpha = 0.05/4 = 0.0125$ ).

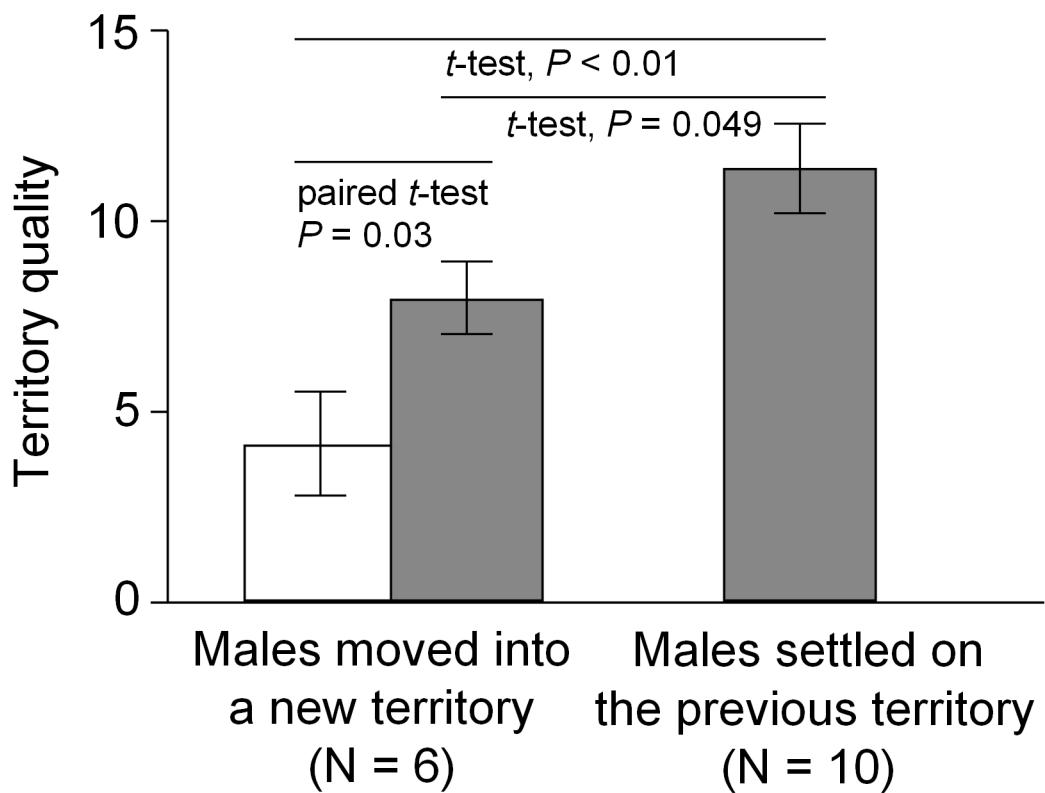
**Table 3.** Mean difference  $\pm$ SD in four male ornaments and territory quality between the two nearest neighbor males (the former breeders – the later breeders within each dyad).

	Raw values	Residuals ornaments <sup>1</sup>
	Difference ( <i>P</i> )	Difference ( <i>P</i> )
All males (10 pairs)		
Throat patch height	1.51 $\pm$ 5.15 (0.38)	1.60 $\pm$ 5.10 (0.35)
Throat coloration <sup>2</sup>	-6.29 $\pm$ 8.89 (0.03)	-2.86 $\pm$ 6.60 (0.10)
Tail length <sup>2</sup>	5.18 $\pm$ 9.00 (0.051)	5.41 $\pm$ 8.90 (0.04)
Size of the white spots in the tail <sup>2</sup>	5.84 $\pm$ 11.25 (0.07)	6.68 $\pm$ 10.38 (0.04)
Territory quality <sup>2</sup>	4.10 $\pm$ 5.72 ( <b>0.025</b> )	—
Matched age-class (6 pairs)		
Throat patch height	0.16 $\pm$ 5.72 (0.95)	0.21 $\pm$ 5.65 (0.93)
Throat coloration <sup>2</sup>	-3.45 $\pm$ 6.19 (0.12)	-1.49 $\pm$ 2.49 (0.10)
Tail length <sup>2</sup>	1.53 $\pm$ 9.59 (0.36)	1.66 $\pm$ 9.33 (0.34)
Size of the white spots in the tail <sup>2</sup>	8.40 $\pm$ 12.15 (0.08)	8.88 $\pm$ 11.25 (0.056)
Territory quality <sup>2</sup>	2.33 $\pm$ 5.85 (0.19)	—

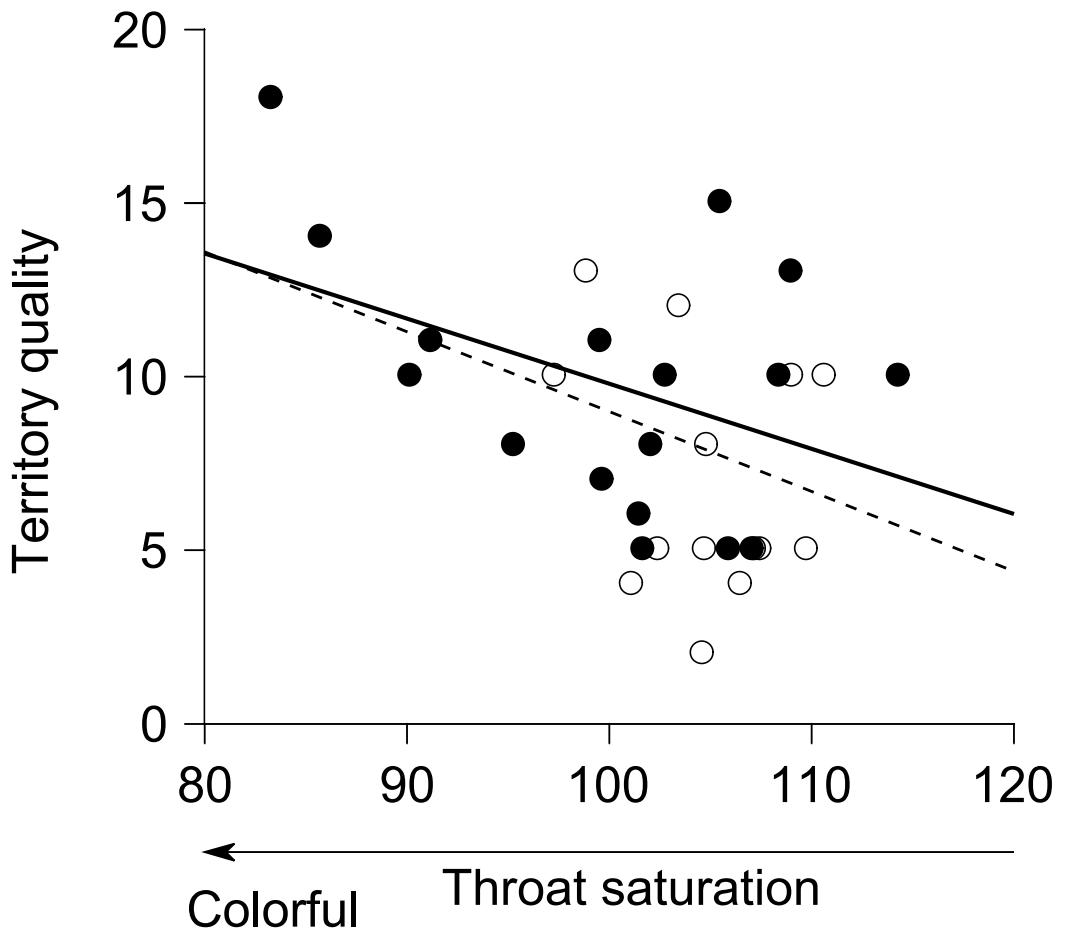
*P*-values were based on paired *t*-test. Bold *P*-values indicate significance after adjustment of multiple comparison (territory quality,  $\alpha = 0.05$ ; four male ornaments,  $\alpha = 0.05/4 = 0.0125$ ).

<sup>1</sup> Residuals from regression of each male ornament to territory quality was used.

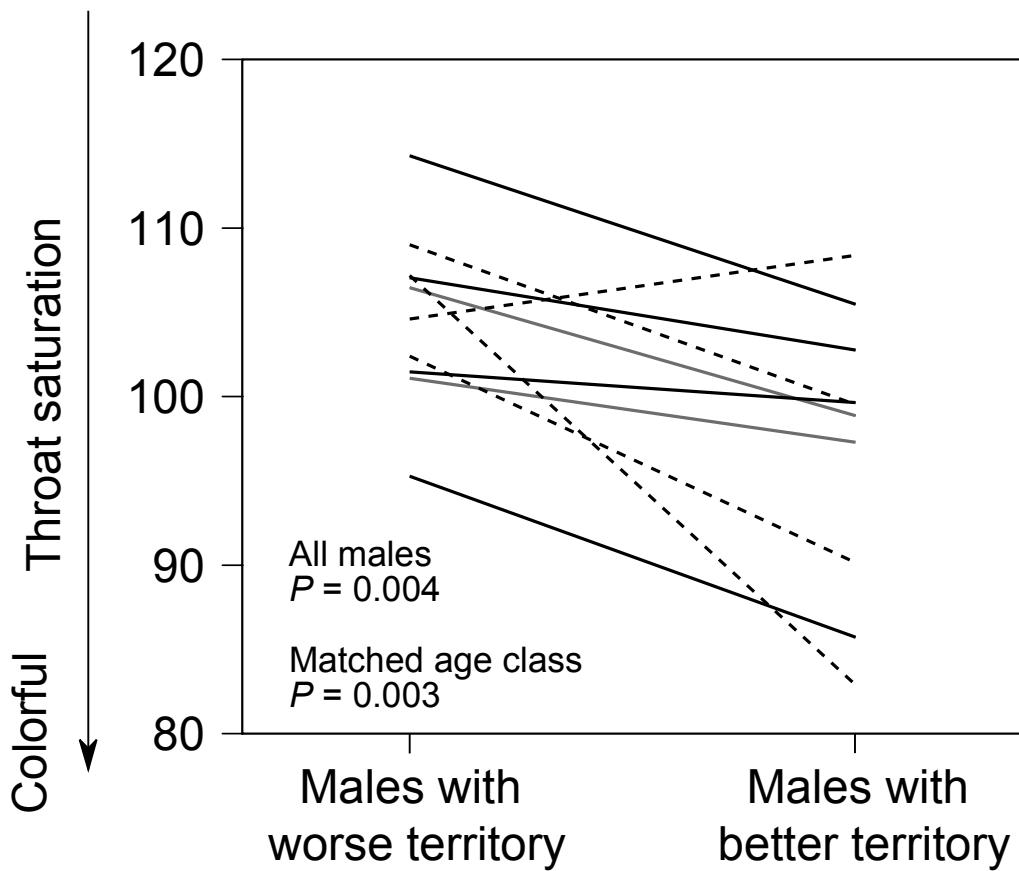
<sup>2</sup> One-tailed tests were used.



**Figure 1.** Territory quality of males that moved into a new territory without their previous territories being occupied and males that settled on the previous territories. White and black bars indicate the mean ( $\pm$ SE) quality of the previous and current territory, respectively.



**Figure 2.** Relationship between territory quality and male throat coloration (filled and open circles indicate ASY and age-unknown males, respectively). Linear regression lines are shown [bold line: all males, territory quality =  $32.41 - 0.023 \times$  throat saturation,  $r = -0.44$ ,  $P = 0.0127$ ; black line: ASY males, territory quality =  $28.50 - 0.18 \times$  throat saturation,  $r = -0.43$ ,  $P = 0.08$ ].



**Figure 3.** Comparing throat coloration between the two nearest neighbor males (solid lines: dyads were both ASY males; hatched lines: ASY males occupied better territories within dyads; gray lines: dyads were both age-unknown males).  $P$ -values were based on paired  $t$ -tests, one-tailed. Use of a one-tailed test was justified by the trends observed in the population (Fig. 2).

**Appendix.** Correlations among body weight and the four ornaments in all males (N = 31) and ASY males (lower column, N = 17) in the current sample.

	BW	TP	TC	TL	WS
Body weight (BW) <sup>1</sup>	—	0.37 (0.07)	-0.19 (0.36)	0.49 (0.01)	0.32 (0.12)
Throat patch height (TP)	-0.18 (0.58)	—	-0.26 (0.11)	0.43 (0.02)	0.48 (<0.01)
Throat coloration (TC)	0.06 (0.84)	-0.41 (0.11)	—	-0.05(0.79)	-0.15 (0.43)
Tail length (TL)	0.41 (0.18)	-0.28 (0.27)	0.20 (0.44)	—	0.57 (<0.01)
Size of white spots in the tail (WS)	0.44 (0.15)	-0.16 (0.54)	-0.22 (0.39)	0.35 (0.17)	—

<sup>1</sup> Sample sizes are reduced to 25 and 12 in all males and ASY males, respectively

# GENERAL DISCUSSION

Despite the numerous examples on complex male ornamentation in natural systems, reasons why animals have developed such complex ornaments remain unclear (van Doorn and Weissing, 2004). The intense debates for 150 years did not bring any conclusion as in the case that peacocks developed complex multiple ornaments in their train (Takahashi et al., 2008), probably due to too many ornaments to determine the exact target of sexual selection, such as the length of tail, the number and/or density of ocelli, brightness, iridescence and so on (Loyau et al., 2008). Species with distinct two ornaments is thus suitable to study why animals got multiple ornaments. Barn swallow males have the ornaments, throat coloration and white spots in the tail.

Although throat coloration and white spots in the tail are shown to be sexually selected, either ornament was studied in respective populations (Kose and Møller, 1999; Kose et al., 1999; Ninni, 2003; Safran and McGraw, 2004). Sexual selection on the other ornament was not concerned. Then, it remained unknown whether or not both ornaments are together sexually selected in a single population, which is necessary to test the obsolete signal hypothesis (Møller and Pomiankowski, 1993). The present study demonstrated that obsolete signal hypothesis could not be applied in barn swallows, because both ornaments were sexually selected even in a single population (Chapter 3).

Sexual selection on two male ornaments can be explained by intersexual selection alone or together with intrasexual selection. Turner (2006) reviewed many supports for intersexual selection but none for intrasexual selection from dense populations. For the balanced discussion about sexual selection and its mechanism, the relative importance of

intersexual selection and intrasexual selection should be argued under sparse population in the base of the current insights.

In the dense populations reported, extra-pair paternity was frequently found (Møller, 1994). Since extra-pair paternity appears to be a result from female choice, the observed high frequency indicated the importance of intersexual selection (Turner, 2006). However, extra-pair paternity was rare and unimportant in the sparse population located at Joetsu city, probably due to low density (Chapter 2). Thus, extra-pair paternity and the intersexual selection are weak in the sparse population.

Møller (1994) argued that male-male competition for territory seemed to be unimportant for sexual selection, based on indirect measure of female choice for territory. In the present study, territory quality influenced on the mating order of its owner (Chapter 4), and was related to the settlement pattern of males (Chapter 5), suggesting that males competed for high-quality territory. Therefore, intrasexual selection through male-male competition for territory needs to be taken into account when studying sexual selection on male ornaments.

Intrasexual selection and intersexual selection acts on throat coloration and white spots in the tail, respectively (Chapter 5). Males with colorful throat held high-quality territory, suggesting intrasexual selection. On the other hand, males with large white spots had mating advantages after controlling for territory quality, suggesting intersexual selection. Since a single ornament is used in intersexual selection, hypotheses based on multiple female choice might be difficult to explain the multiple ornaments in this species. Alternatively, multiple receiver hypothesis, in which some ornaments are used in intersexual

selection and the others are used in intrasexual selection (Andersson et al., 2002), was proposed (Chapter 5).

Mating advantage in relation to male ornamentation has been assumed to be the result of female choice for the ornaments, although it is affected by male–male competition indirectly through the territory quality (Wiley and Poston, 1996; Tarof et al., 2006). Even when multiple ornaments were related to mating advantage, the relative importance of intersexual selection and intrasexual selection were different for each ornament, as predicted by multiple receiver hypothesis. When considering that intrasexual selection and intersexual selection are often difficult to be decoupled in natural systems (Wiley and Poston, 1996), multiple receiver hypothesis would be much more important mechanism for explaining multiple ornaments.

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