

ASSESSMENT OF THE SIGNAL VALUE OF A PLUMAGE ORNAMENTATION  
IN GREAT TITS *PARUS MAJOR* IN TERRITORIAL MALES USING 3D  
PRINTED MODELS

A THESIS SUBMITTED TO  
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES  
OF  
MIDDLE EAST TECHNICAL UNIVERSITY

BY

ALİCAN AVŞAR

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR  
THE DEGREE OF MASTER OF SCIENCE  
IN  
BIOLOGY

AUGUST 2022



Approval of the thesis:

**ASSESSMENT OF THE SIGNAL VALUE OF A PLUMAGE  
ORNAMENTATION IN GREAT TITS *PARUS MAJOR* IN TERRITORIAL  
MALES USING 3D PRINTED MODELS**

submitted by **ALİCAN AVŞAR** in partial fulfillment of the requirements for the degree of **Master of Science in Biology Department, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar  
Dean, Graduate School of **Natural and Applied Sciences**

\_\_\_\_\_

Prof. Dr. Ayşegül Gözen  
Head of Department, **Biology**

\_\_\_\_\_

Prof. Dr. C. Can Bilgin  
Supervisor, **Biology, METU**

\_\_\_\_\_

Assist. Prof. Dr. Çağlar Akçay  
Co-supervisor, **Psychology, Koç University**

\_\_\_\_\_

**Examining Committee Members:**

Assist. Prof. Dr. R. Ian Bowers  
Psychology, Bilkent University

\_\_\_\_\_

Prof. Dr. C. Can Bilgin  
Biology, METU

\_\_\_\_\_

Prof. Dr. Mehmet Somel  
Biology, METU

\_\_\_\_\_

Date: 31.08.2022

**I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.**

Name, Surname: Alican Avşar

Signature :

## ABSTRACT

### ASSESSMENT OF THE SIGNAL VALUE OF A PLUMAGE ORNAMENTATION IN GREAT TITS *PARUS MAJOR* IN TERRITORIAL MALES USING 3D PRINTED MODELS

Avşar, Alican

M.S., Department of Biology

Supervisor: Prof. Dr. C. Can Bilgin

Co-Supervisor: Assist. Prof. Dr. Çağlar Akçay

August 2022, 51 pages

Both genetic and environmental factors determine coloration of bird feathers. Great tits, *Parus major*, have several characteristics controlled by genetics and environment regarding ornamentation. The black tie is one of them regarding the area, width, and coloration, and the black color of the feathers is due to the melanin pigment.

Several lines of evidence suggest that the black tie of great tits has a function in signaling. Great tits with larger black ties were shown to be more active in nest defense, and they performed closer approaches to the intruder in case of an encounter. Furthermore, black tie size was shown to be a signal of dominance between males, and it brought priority to achieve food sources in wintering flocks.

In the present study, we evaluated the role of the black tie in territorial interactions between males during breeding season when birds have already established territories. For this purpose, we performed playback experiments using 3D printed great tit models with narrow and wide ties as well as a negative control (a monochrome gray 3D model). This latter condition was to confirm that the models painted as great tits

are indeed perceived as conspecific individuals.

Our results showed that there is no overall difference in aggression with respect to tie area of 3D models, suggesting that black tie might not function as a signal in territorial interactions. Surprisingly, our control model did not elicit a significantly different aggressive response than the great tit models. This suggests caution in the interpretation of the results and underlines the need to include controls in experimental designs with 3D models.

Keywords: black tie, ornaments, aggression, territory, Paridae, bird song

## ÖZ

### **BÜYÜK BAŞTANKARALARDA *PARUS MAJOR* BİR TÜY SÜSLEMESİNİN SİNYAL DEĞERİNİN BÖLGESEL ERKEK BİREYLERDE 3B YAZDIRILMIŞ MODELLER KULLANILARAK DEĞERLENDİRİLMESİ**

Avşar, Alican

Yüksek Lisans, Biyoloji Bölümü

Tez Yöneticisi: Prof. Dr. C. Can Bilgin

Ortak Tez Yöneticisi: Dr. Öğr. Üyesi. Çağlar Akçay

Ağustos 2022 , 51 sayfa

Kuş tüylerinin rengi hem genetik hem de çevresel faktörlerle belirlenmektedir. Büyük Baştankaralar, *Parus major*, tüy süsleriyle ilgili kalıtsal ve çevresel olarak düzenlenen çeşitli özelliklere sahiptir. Siyah kravat alan, genişlik ve renk açısından bu özelliklerden biridir. Tüylerin siyah rengi melanin pigmentinden kaynaklanmaktadır.

Büyük Baştankaraların siyah kravatının sinyal vermede bir işlevi olduğu önceki çalışmalar aracılığıyla gösterilmiştir. Daha büyük siyah kravata sahip olan Büyük Baştankaraların yuva savunmasında daha aktif olduğu ve bir karşılaşma durumunda davetsiz misafirlere daha yakın mesafeden tepki verdiği bulunmuştur. Ayrıca, siyah kravat büyüklüğünün erkekler arasındaki baskınlığın bir işareti olduğu ve kışlayan kuşlarda besin kaynaklarının elde edilmesinde öncelik sağladığı ortaya konmuştur.

Bu çalışmada, üreme döneminde olan ve savunaklarını çoktan oluşturmuş erkek bireyler arasındaki bölgesel etkileşimlerde siyah kravatın rolünü değerlendirmeyi hedefledik. Bu amaçla, dar ve geniş kravata sahip üç boyutlu (3B) Büyük Baştankara modellerinin yanı sıra bir negatif kontrol (tek renkli bir gri 3B model) kullanarak şarkı deneyleri gerçekleştirdik. Bu kontrol unsuru ile Büyük Baştankara gibi boyanmış modellerin gerçekten de kendine özgü bireyler olarak algılandığının doğrulanmasını amaçladık.

Bu çalışma sonucunda, farklı kravat alanına sahip 3B modellere karşı agresiflik açısından genel bir fark saptanmamıştır. Bu sebeple, siyah kravatın bölgesel etkileşimlerde işlevsel olmayabileceği öngörülmektedir. Beklenenin aksine, negatif kontrole (gri 3B model) karşı gösterilen agresiflik davranışı ile büyük baştankara modellerine gösterilen agresiflik arasında anlamlı bir fark görülmemiştir. Çalışmanın sonuçları değerlendirilirken bu durum göz önünde bulundurulmalıdır. Ayrıca bu durum, 3B modellerin kullanıldığı çalışmalarda kontrol unsurunun dahil edilmesinin gerekliliğini ortaya koymaktadır.

Anahtar Kelimeler: siyah kravat, tüy süsleri, agresiflik, savunak, Paridae, kuş şarkısı



*To all the street animals of the world*

## ACKNOWLEDGMENTS

There are no words to express my gratitude to my supervisor Prof. Dr. C. Can Bilgin for his guidance and encouragement, and to my co-supervisor Asst. Prof. Dr. Çağlar Akçay for his criticism and for standing by my side in my challenging moments.

I would like to extend my sincere thanks to my thesis committee members Prof. Dr. Mehmet Somel and Assist. Prof. Dr. Robert Ian Bowers for their valuable feedback.

I would like to thank TÜBİTAK for their support through BİDEB-2210A program.

I am deeply indebted to Assoc. Prof. Dr. Ulaş Yaman for printing the 3D models, Sude Uysaler for painting them, and Buse Uysaler for her companionship and endless support. I am extremely grateful to Iğın Ertan Gürol for participating in the field experiments, friends from our laboratory Selen Akçakoca, Mustafa Cırık, Elif Göçer and Mert Kükrer for their help, my dear friend Ufuk Sarısaltık for hosting me during the field work, and Ümran Köken, Şükran Köken and Papi for opening their doors to me, and İnci Gökmen and Ali Gökmen for their heartfelt support.

For their great support, I am deeply grateful to my friends Esmâ Demirel, Ezgi June Olgaç, Dudu Erol, Ekin Yılmaz, Serkan Atalay, and Sinem Kurtar who stood by me in my difficult times during this work.

I would like to thank all the great tit birds involved in my work and our revolutionary tradition that keeps this campus green.

I am immensely thankful to my mother Kibriye Avşar, my father Hüseyin Avşar, my brother Akın Avşar and my uncle İsmail Korkmaz for all the sacrifices they've made, and for supporting me even in their most difficult times.

Finally, very special thanks to my one and only, lovely, and spicy Aslanboncuk, a miracle of life to me. My love and devotion to him is immeasurable.

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## **LIST OF ABBREVIATIONS**

3D	3 Dimensional
SSH	Status Signaling Hypothesis
RHP	Resource Holding Potential
STI	Simulated Territorial Intrusion

## CHAPTER 1

### INTRODUCTION

#### 1.1 Feather Ornamentations

Visual ornaments are secondary sex traits with a wide array of functions across species both in intra- and inter-sexual selection contexts [Enbody et al., 2017] and they have a remarkable function in communication including signals towards conspecifics and predators [Butcher and Rohwer, 1989]. With the advancements of chromatography tools and chemistry techniques, knowledge about pigments has started to improve and new questions about bird coloration have become prominent [Brush, 1990]. Birds have been favorite subjects in the exploration of ornaments since many bird species display a diverse set of colorful, bright, and conspicuous ornaments [Jawor and Breitwisch, 2003].

Ornaments can transmit information about the owner's quality when there is a cost associated with it [Hill, 1991, Zahavi, 1975] and the cost may be physiological and indicate signal honesty, in cases like [e.g., the peacock's tail] [Enbody et al., 2017]. Such transmission of information attributed to quality might be various, and examples include parental abilities such as nest attendance [Hill, 1991], nest building [Broggi and Senar, 2009], foraging ability [Senar et al., 2002, Senar and Escobar, 2002], habitat quality and condition [Slagsvold and Lifjeld, 1985, Ferns and Hinsley, 2007]; health status such as parasite load [Thompson et al., 1997, Fitze and Richner, 2002], immunocompetence [Saks et al., 2003], male viability [Hill, 1991], and cognitive abilities such as problem-solving skills [Mateos-Gonzalez et al., 2011].



Figure 1.1: Examples of conspicuous plumage patterns. Clockwise from top to left: TheOtherKev, Allanlau2000, GKorovko, GeorgeB2, Daniel Lowth, Kev on Pixabay.com.

## 1.2 Coloration of Feathers

Plumage coloration can originate due to structural characteristics of feathers, pigment molecules or their combination [Galván and Solano, 2016], and the main variation of avian plumage coloration is a result of two pigments, carotenoids and melanins, which are extensively found in birds [Brush, 1990, Galván and Solano, 2016, Jawor and Breitwisch, 2003]. Enzymatic alteration of carotenoids adds to the variation in coloration and interactions between proteins and pigments might even increase diversity of coloration [Brush, 1990].

Carotenoids are responsible for red and yellow colorations [Brush, 1990, Jawor and Breitwisch, 2003] and yellow flank feathers of great tits (*Parus major*) are an example of carotenoid-based plumages (see Figure 1.2). Although birds cannot synthesize carotenoids de novo [Brush, 1990, Fitze and Richner, 2002], they are able to modify carotenoids in diverse ways through enzymatic regulations [Brush, 1990]. Plants and insects are the major sources of carotenoids for birds [Brush, 1990] and they involve in pivotal physiological processes [Jawor and Breitwisch, 2003]. Consequently, carotenoid-base plumages can indicate the quality of individuals and territories in re-

lation to foraging abilities and food availability respectively [Brush, 1990, Slagsvold and Lifjeld, 1985] and as a result, carotenoids have become a popular subject of research about individual and territorial quality [Jawor and Breitwisch, 2003] (also see [Ferns and Hinsley, 2007, Hill, 1991]).

In contrast to carotenoids birds can synthesize and deposit in the feather follicle another pigment molecule, melanins [Brush, 1990]. Black and brown colorations are due to melanin pigmentation [Brush, 1990, Jawor and Breitwisch, 2003] and melanin also contributes to endurance against abrasion [Brush, 1990]. Melanin is primarily associated with social interactions and aggressive behavior rather than individual condition [Jawor and Breitwisch, 2003, McGraw and Hill, 2000, Senar, 1999]. The ventral black tie of great tits is an example of melanin-based ornament (see Figure 1.2).

Ornament production is an energetically costly process that requires the involvement of enzymes, ions, hormones, and nutrition, although the costs differ between the type of pigment used. In birds, melanin pigments are thought to be relatively cheap to produce while carotenoid pigments are environmentally limited and thus may be a reliable indicator of the resource acquisition potential of the bearer. Thus, carotenoid ornaments are mainly associated with foraging, nutritional condition and health status regarding endoparasites and immunity. On the other hand, melanin is mainly associated with aggression, dominance, and intersexual competition [Jawor and Breitwisch, 2003]. Given the relatively cost of the melanin production what maintains the reliability of melanin-based signals has been a topic of interest for several decades [Rohwer, 1975]. One hypothesis is the Status Signaling Hypothesis (the SSH) which is centered around the relationship between conspicuous signals and their signaling function to signal status and establish the hierarchy.

### 1.3 The Status Signaling Hypothesis

Conspicuous signals such as feather ornaments enable individuals to overcome conflicts with low-cost solutions instead of physical engagements between individuals, and they facilitate the evaluation of the opponent's resource holding potential [Fitze and Richner, 2002]. This hypothesis, known as the status signaling hypothesis (SSH), states that the plumage variability evolved to function in intraspecific competition for establishment and maintenance of a social dominance hierarchy within members of wintering flocks [Rohwer, 1975]. Subordinates carrying smaller badges can avoid costly interactions with dominants while dominants with their larger badges can lower their effort to preserve status quo [Rohwer and Rohwer, 1978, Rohwer, 1982, Senar and Camerino, 1998, Philip Whitfield, 1987] and newcomers can merge into established flocks conveniently avoiding test fights [Rohwer, 1975]. The reason for why such status badges are signals of dominance is their correlation with resource holding potential (RHP) or fighting abilities of individuals.

The SSH was built on the behavior and dominance hierarchy in wintering flocks of birds, and it primarily explains the correlation between status badges (ornaments) and priority for resources with respect to dominance hierarchy [Rohwer, 1975]. In accordance with this, it was shown that plumage variability is observed in wintering flocks with variable stability where individuals join and leave flocks, and social dominance is the key in the competition over resources [Rohwer, 1975]. On the other hand, monomorphism in plumage is observed in territorially dispersed species where the necessity of such variability is low in this context [Rohwer, 1975].

Characteristics with respect to within-group interactions of a population is a strong determinant for the emergence and sustainability of status badges in that population [Rohwer, 1982]. Three requirements were emphasized for conspicuous badges to emerge [Rohwer, 1982]. First, a population should experience aggressive competition over resources. Second, individuals should have variable fighting abilities or RHP which will be transmitted by status badges. Third, frequent confrontations between rivals should exist while individual recognition is not easy. This can be a result of living in large and unstable flocks where individuals join and leave frequently. When these criteria are fulfilled, emergence of a status badges are favored [Rohwer, 1982].

### 1.3.1 Status Signaling in Great Tits

One of the species where SSH was tested extensively is the great tit. Great tits possess a plumage ornament, the black tie (see Figure 1.2) that is a potential candidate to be a status badge given the remarkable variation in the size of black tie [Riyahi et al., 2014]. By this variation, great tits were hypothesized to be able to capture and represent the variation in RHPs. As a result, black tie was proposed to be a status badge that signals bearer's RHP or fighting abilities. Second, great tits display a social dominance hierarchy in winter flocks that determines the priority for resources. Third, great tits live in unstable flocks in winter [Järvi and Bakken, 1984] and a status signal is more favorable under this condition [Rohwer, 1975]. Therefore, the species shows a good fit to conditions for the emergence of status signaling [Rohwer, 1982]. The presence of status signaling in great tits was shown by several studies [Järvi and Bakken, 1984, Järvi et al., 1987, Lemel and Wallin, 1993, Maynard Smith and Harper, 1988, Pöysä, 1988] with various approaches such as cage experiments, tie enlargement, dummy birds, and behavioral alterations by testosterone.



Figure 1.2: Male (left) and female (right) great tit (*Parus major*) individuals. Images are from Wikimedia Commons.

While the evidence supports that the black tie of great tits functions as a status badge to resolve the conflicts in wintering flocks it is not clear whether black tie also has a function during the breeding season when great tits are territorial. As noted above, the SSH suggests that badges of status are primarily functional in larger flocks with frequent changes in membership, while most territorial systems, including the breeding season territories of great tits are characterized by stable relationships between small number of neighbors. Nevertheless, great tits show the same variation in tie area during the breeding season as in winter flocks (great tits only molt once a year and after the breeding season, [Orell and Ojanen, 1980]). As a result, the black tie may still retain some signaling function during the breeding season.

#### **1.4 Aim of This Thesis**

The aim of this study is to investigate whether the black tie of great tits functions as a signal in intraspecific competition. Previous research investigated the same question showed that black tie is a potential signal of dominance in wintering flocks. Whether this function retains when great tits become territorial is unknown. To answer this question, we carried out simulated territorial intrusions where we coupled playback of great tit songs with 3D printed models of great tits with different sized black ties painted on them. We reasoned that if the black tie size is a signal of aggression or dominance in territorial interactions, resident males would respond with higher aggression to the models with larger black ties. A second aim of our study was to evaluate the effectiveness of using 3D printed models of birds in behavioral research. While 3D printed models afford standardization of shape and can be produced in larger quantities, very few studies examined how they are perceived by the actual subjects [Behm et al., 2018, Bentz et al., 2019, Chen et al., 2022]. To answer this second question, we also presented subjects with a 3D printed model that was painted in monochrome gray as a control condition. We predicted that birds would respond more strongly to models painted like a great tit than painted in monochrome gray.



## CHAPTER 2

### MATERIALS & METHODS

#### 2.1 Study Species

Great tits (*Parus major*) are wide-spread species that has been extensively studied as a model system in evolutionary and behavioral ecology. The distribution of great tits starts from the Western Europe and extends across Asia [Johansson et al., 2013]. They are territorial in breeding season, but form flocks in the winter. They prefer mixed deciduous forests, but it is observed that a wide range of habitat including pine forests and even urban areas with vegetation host great tits [Kavak, 2015].

Great tit plumage is characterized by yellow flank feathers on their chest and ventral black tie from head to tail (see Figure 1.2). Females and males differ in the size of black tie (see [Riyahi et al., 2014]), and they can be distinguished even from distant observations up to 20-30 m. Great tits are highly abundant on METU Campus (where this study was conducted), and they can be found in urban and rural parts of the campus area. They are easily located and differentiated with the aid of their distinct visual and vocal characteristics. Nest boxes located in the forest and cavities in human made structures serve them as nesting opportunities. These opportunities enable us to locate great tits and carry out experiments with them as well.

## 2.2 Study Site and Subjects

This study was conducted on the Middle East Technical University Campus located in Ankara, Turkey. Trials were held in Spring 2021 between 17 April and 2 May. METU Campus has a large forest area being artificially afforested mainly by pine trees since 1960. Nest opportunities for great tits are primarily the nest boxes distributed throughout the forest, natural cavities, and cavities in human-made structures (e.g., buildings, lamp posts), and this enables to monitor populations in both rural and urban habitats. Our study included 20 male subjects and at least 15 of these individuals were observed to be paired with a female. It is uncertain whether the rest five individuals were paired with a female.

Individuals with known nest ( $n=11$ ) were subjected to territorial intrusions 5 m away from their nest. For the subjects with unknown nest location ( $n=9$ ), we detected the territory center by observing the subjects in preceding days of trials. These individuals were inhabiting close to buildings in the urban parts of the campus. Birds inhabiting neighboring territories were tested on different days, and we ensured that subjects are out of earshot of any trial on the same day (at least 500 m away).



Figure 2.1: Google Earth image of METU Campus with each individual's territory is marked with blue pins.

### 2.3 Nest Boxes

On METU Campus, nest boxes are used for longstanding monitoring of several species such as great tits (*Parus major*), coal tits (*Parus ater*) (see [Kavak, 2015]) and scops owl (*Otus scops*) (see [Göçer, 2018]). Eleven subjects from our study group were occupying nest boxes. Seven of these individuals were habiting small nest boxes (13.5 cm x 13.5 cm (width) x 12 cm (depth)) (Figure 2.2). Small nest boxes were 2-2.5m above ground on average (see [Kavak, 2015] for details). Four individuals were habitating large nest boxes (22 cm x 24 cm (width) x 32,5 cm (depth)) (Figure 2.2) which are placed for scops owl species monitoring and these boxes were 2.5-3 m above ground on average (see [Göçer, 2018] for details). In bird populations in which identification rings are not present such as ours, nest boxes become highly important when multiple trials are needed with same individuals.



Figure 2.2: Two types of nest boxes used in this study.

## 2.4 Playback Stimuli and Recordings

Previous recordings of male great tits from Spring 2018 and 2019 were used to prepare playback stimuli to perform simulated territorial intrusions (STIs). These recordings were recorded using Marantz PMD660 or PMD661 recorders with the aid of a shotgun microphone (Sennheiser ME66/K6). Spectrograms of the recordings were examined in Syrinx software (John Burt, Seattle, WA). We chose male songs of about 3-seconds length with good signal-to-noise ratios. For each song, we filtered low-frequency (<1000 Hz) noise and added a silent period to create a seven second loop that contained a single song that was repeated to make a five-minute stimulus tape. We used a different stimulus from distinct individuals for each subject. The stimulus song for each subject came from an individual that was at least one km away from the subject.

## 2.5 Model Preparation

We used 3D printed and hand painted great tit models to represent territorial intruders. The stl file used for printing can be found as a supplementary material. We painted four narrow tie area (300 mm<sup>2</sup>) and four large tie area (475 mm<sup>2</sup>) models based on the measurements of previous work (see [\[Riyahi et al., 2014\]](#) ). These measurements were representing the lower and upper boundaries of the tie area distribution of males. Thus, narrow tie models represent males with very low RHP and status so that they are very likely to be a subordinate to almost all other males in a flock. In contrast, wide tie models represent males with quite high RHP and status, eventually, they are dominant to almost all other males in a flock.

By having replicates, we wanted to decrease any effect due to any model. We painted an additional model in monochrome gray to use as a negative control for ensuring the perception of great tit models as conspecifics. Because the 3D printing ensures faithful replication of the shape of the model and the fact that we only painted the model in a uniform color, we did not have replicates of the gray model. Models were produced with Ultimaker 3 Extended 3D printer and Ultimaker PLA Filament (2.85 mm) was used as raw material.



Figure 2.3: 3D printed control, narrow and wide tie models used in experiments.

## 2.6 Procedure

In this study, 3D models were used with playback stimuli, and we performed randomized controlled trials for each subject. Each trial consisted of ten minutes, corresponding to five minutes of playback and a subsequent five min silent period. We randomly determined i) the replicate to be used (4 narrow tie, 4 wide tie), ii) sequence of trials (control, narrow, wide) and iii) the stimulus to be used for each subject before experiments. Each subject was tested with small tie, large tie and control models using the same song stimuli ( $n=20$  subjects  $\times$  3 = 60 trials in total) on consecutive days or with 1-2 day breaks due to weather conditions and nonattendance of subjects on some occasions.

We placed a Bluetooth speaker (JBL Go 2) on a tree branch that is 1.5 m high from the surface and five meters from the nest. Then, we positioned the 3D model on the speaker, facing towards the nest entrance. In cases, where we did not know the nest location, we used a central position in the territory as described above. We then placed markers at 0.5 m, 1 m, and 5 m, to help with the estimation of the distance of subject to speaker during the trial.

We used a smartphone to control the playback remotely via Bluetooth, standing about 20 m from the speaker. We initiated the playbacks after the speaker and the model was setup and continued until the subject entered a five m radius of the model. This was done to ensure that the model was seen by the subject. We continued the playback for five minutes after this criterion was reached and recorded the vocal and physical

activity of the males using a voice recorder and a shotgun microphone. When targets attacked the model, we stopped the playback considering a conflict would have ended in these circumstances. An observer narrated the flights of the subject, distance to the model and specified the vocal elements of the subject.

## 2.7 Response Measures

We scanned and annotated the trial recordings in Raven Pro (Version 1.6.1) and extracted the following response measures: songs, flights, and distance to the speaker after each flight or movement. We extracted the response measures for the five-minute playback period after the subject entered the five m radius of the model. Then, we calculated song rate (song per min), flight rate (flight per min), proportion of time spent within 1m and closest approach to the model (m). Previous studies found that song rate is generally negatively aggressive behaviors in this species ( [Akçay et al., 2020, Hutfluss et al., 2021]).

Behaviors like flight and approach are not necessarily aggressive signals but considered as aggressive behaviors, and in the context of aggressive encounters, these behaviors do allow us to describe and quantify aggression [Akçay et al., 2014]. Their potential of measuring aggression is a result of their evident association with a probable attack which is the ultimate resolution of a conflict, and this association is apparently a consequence of the necessity of physical intrusion to pave the way for an attack [Akçay et al., 2014]. Many studies adopting the use of spatial measures point out the consensus around their significance in the assessment of aggression (e.g, [Hardman and Dalesman, 2018, Searcy et al., 2006, Searcy and Beecher, 2009, Vehrencamp et al., 2007]). Moreover, in studies using dummies, these measures have been shown to predict attack efficiently ( [Akçay et al., 2013, Akçay et al., 2014, Araya-Ajoy and Dingemanse, 2014, Ballentine et al., 2008, Hof and Hazlett, 2010, Searcy et al., 2006]).

## 2.8 Data Analysis

Considering the high correlation between time spent within 1 m of the model, closest approach, and flight rate (Table 3.1, Figure A.1, A.2, A.3 in Appendix A), we performed a principal component analysis, and achieved a single measure of aggression. Principal component 1 explained 75% of variance and we used it as aggression scores in further analysis. Also, PC1 correlated positively with time spent within 1 m and flight rate, and negatively with closest approach (Table 3.1, see also B.1, B.2, B.3 in Appendix B).

We observed that the responsiveness of the subjects decreased in each successive trial (Figure 3.2). To find out such relationship between aggression scores (PC1) and trial order (1st, 2nd, 3rd), we used a linear mixed model (LMM). Aggression scores were used as response measures while trial order was the fixed factor, and subject ID is a random effect. Since aggression scores of the 3rd trial were significantly different lower than those of the 1st trial, we added trial order as independent factor in LMMs in the following analysis to be able to capture any interaction of other variables (treatment, song rate) with trial order.

We used another LMM to analyze the relationship between aggression scores (PC1) and experimental treatment (large tie, narrow tie, and control). Aggression scores were taken as response measures while treatment and trial order were fixed factors, and subject ID is a random effect. We used an analogous LMM for song rates as response variable.

## 2.9 Ethical Note

All experiments included in the present study are consistent with ASAB/ABS guidelines for the treatment of animals in behavioral research and teaching. Great care was taken to minimize the presence and disturbance in the territories, and experiments were kept around 10-15 minutes (including five minutes of playback). Avoidance of invasiveness was considered as high priority and no subjects were captured or handled throughout experiments.





## CHAPTER 3

### RESULTS

#### 3.1 Attack Counts and PCA

In the investigation of whether the black tie of great tits is used as a signal in territorial interactions, we recorded the aggressive behavior of subjects ( $n=20$ ) via STIs using 3D great tit models of different tie size (narrow & wide). Our data include flight rate (flight per min), proportion of time spent within 1m, closest approach (m), song rate (song per min) and attack occurrences and counts (if present).

Table 3.1: Spearman's rank correlation coefficients (P-values) for the correlation between aggression measures and PC1 with loading coefficients for the PC1 of their PCA ( $n=20$ ). There are three observations for each subject with respect to treatment (control, narrow, wide), and 60 data points were included in the below analysis.

	<b>Time spent within 1 m</b>	<b>Flight rate</b>	<b>Closest approach</b>
<b>Time spent within 1m</b>	-	0.72 (<0.01)	-0.84 (<0.01)
<b>Flight rate</b>	-	-	-0.72 (<0.01)
<b>PC1</b>	0.93 (<0.01)	0.87 (<0.01)	-0.89 (<0.01)
<b>Loading coefficients</b>	0.89	0.88	-0.82

Keiser-Meyer-Olkin measure = 0.73; Bartlett's Test: Chi-square = 197.1, p-value <0.001

Birds that eventually attacked the model also had significantly higher aggression scores ( $p<0.05$ , Figure [3.1](#)). Six attacks were recorded from three individuals (3 from one, 2 from another, 1 from the last individual) while 54 of 60 trials ended without any attack. Among these attacks, three attacks to narrow, two attacks to wide and one attack to control model were recorded.

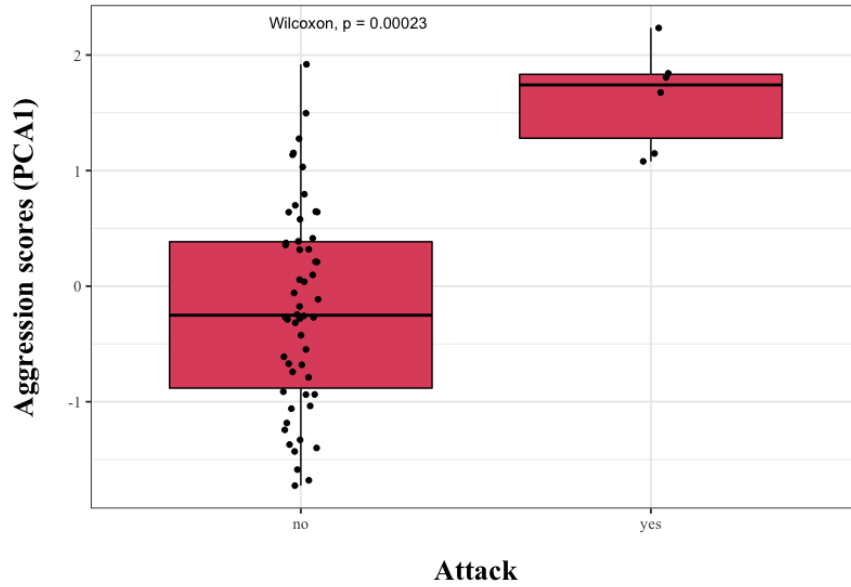


Figure 3.1: Aggression scores distributed according to occurrence of attack. Wilcoxon Sum Rank Test (Mann-Whitney U Test) showed that the difference in aggression scores (PC1) is significant between two circumstances (attack or no attack) ( $p < 0.05$ ).

### 3.2 Order Effect

We analyzed the LMM (Table C.1 in Appendix C) including aggression scores (PC1) as response variables and trial order as fixed factor with ANOVA (type III tests). This analysis revealed that a significant difference exists in aggression scores between at least two groups ( $p < 0.05$ , Table 3.2).

Table 3.2: ANOVA table (Type III tests) for LMM of trial order. Aggression scores were taken as response variables and trial order was the fixed factor. Subject ID was the random factor in the model (pairwise effect sizes - Cohen's  $d$  ( $\pm se$ ) :  $trial1 - 2 = 0.50(\pm 0.32)$ ,  $trial1 - 3 = 0.93(\pm 0.32)$ ,  $trial2 - 3 = 0.43(\pm 0.32)$ )

	Wald Chisq	Df	Pr(>Chisq)
<b>Intercept</b>	2.34	1	0.126
<b>Trial</b>	8.65	2	0.013*

\* $P < 0.05$

Post-hoc test (Tukey’s HSD) (Table 3.3) showed that aggression scores of 3rd trials are significantly lower than those of the 1st trials ( $p < 0.05$ ) and there is not a significant difference in aggression scores between 1st – 2nd and 2nd – 3rd trials ( $p > 0.05$ ). However, an overall decrease in aggression scores (PC1) is observed as trials proceed (Figure 3.2).

Table 3.3: Post-hoc analysis (Tukey’s HSD) of LMM of trial order with aggression scores as response measures and trial order as the fixed factor.

Contrast	Beta Estimate	Std.Error	Df	t-value	p-value
1-2	0.35	0.223	38	1.57	0.27
1-3	0.66	0.223	38	2.94	0.015 *
2-3	0.3	0.223	38	1.37	0.37

\* $P < 0.05$

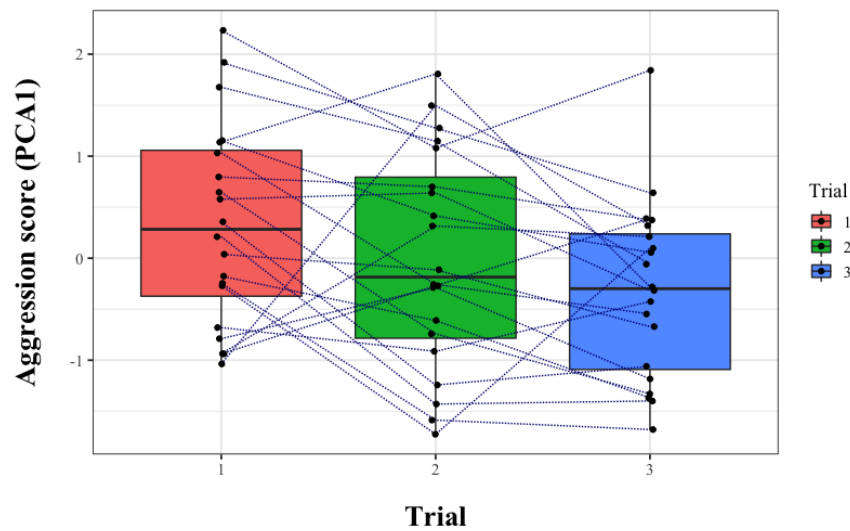


Figure 3.2: Aggression scores (PC1) with respect to trial order ( $n=20$ ). Dotted lines link the aggression scores of an individual obtained during each trial.

### 3.3 The Relationship Between Treatment and Aggression

We analyzed the LMM including aggression scores (PC1) as response variables (Table D.1 in Appendix D), and treatment (narrow, wide, control) and trial order as fixed factors with ANOVA (type III tests, Table 3.4, also see Figure 3.4). Analysis showed that there is no significant difference in aggression scores between treatment groups (control, narrow wide) ( $P=0.66$ ). In other words, there is no evidence for an effect of treatment on aggression. Besides, the interaction between treatment and trial order did not yield any significant difference (see Table E.1 in Appendix E for comparison).

Table 3.4: ANOVA table (Type III tests) for LMM of aggression scores (pairwise effect sizes in Cohen's  $d$  ( $\pm se$ ) :  $control - narrow = 0.26(\pm 0.33)$ ,  $control - wide = -0.008(\pm 0.32)$ ,  $narrow - wide = -0.27(\pm 0.39)$ ).

	Wald Chisq	Df	Pr(>Chisq)
<b>Intercept</b>	2.2	1	0.14
<b>Treatment</b>	0.84	2	0.66
<b>Trial</b>	8.56	2	0.014*

\* $P < 0.05$

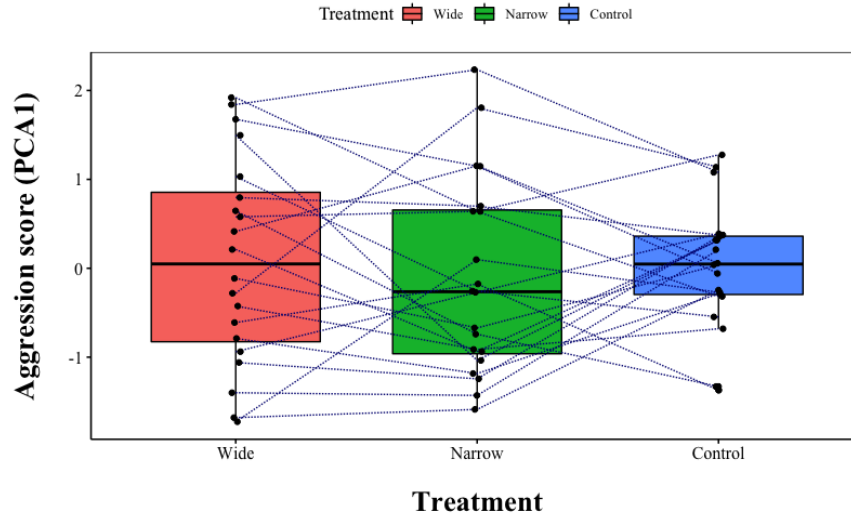


Figure 3.3: Aggression scores (PCA1) with respect to conditional treatments (n=20). Dotted lines link the aggression scores of an individual obtained under each treatment.

### 3.4 Song Rate

#### 3.4.1 The Effect of Treatment on Song Rate

We analyzed the LMM including song rate as response variables, and treatment and trial order as fixed factors (Table F.1 Appendix F) with ANOVA (type III tests, Table 3.5, also see Figure 3.4). Analysis showed that there is no significant difference in song rate between treatment groups (control, narrow & wide) ( $P=0.37$ ). The interaction between treatment and trial order did not yield any significant difference (see Table G.1 in Appendix G for comparison).

Table 3.5: ANOVA table (Type III tests) for LMM of song rate (pairwise effect sizes Cohen's  $d (\pm se)$  :  $control - narrow = -0.1(\pm 0.33)$ ,  $control - wide = 0.34(\pm 0.32)$ ,  $narrow - wide = 0.43(\pm 0.33)$ ).

	Wald Chisq	Df	Pr(>Chisq)
<b>Intercept</b>	74.93	1	<0.01
<b>Treatment</b>	2.00	2	0.37
<b>Trial</b>	3.72	2	0.16

\* $P < 0.05$

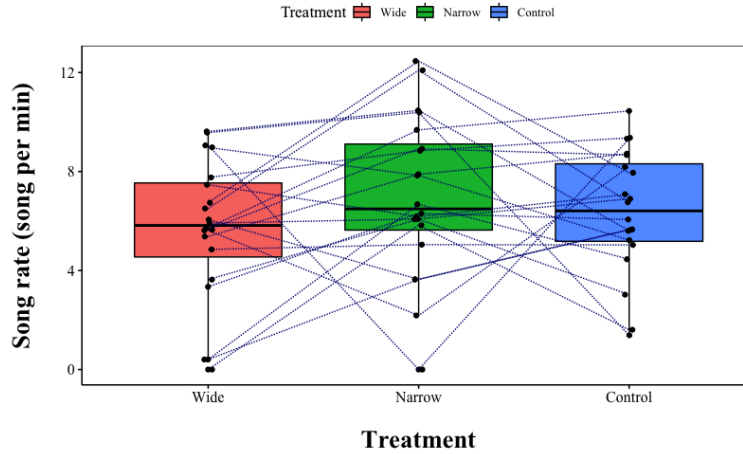


Figure 3.4: Song rate (song per min) with respect to conditional treatment (n=20). Dotted lines link the song rate of an individual obtained during each trial.

### 3.4.2 Relationship Between Song Rate and Aggression

The relationship between aggression scores (PC1) and song rate modified by treatment. It was observed that aggression scores are negatively correlating with song rate under narrow tie ( $p < 0.05$ ) and wide tie ( $p < 0.05$ ) treatments. However, their correlation is reversed under control treatment ( $p < 0.05$ ) (Figure 3.5).

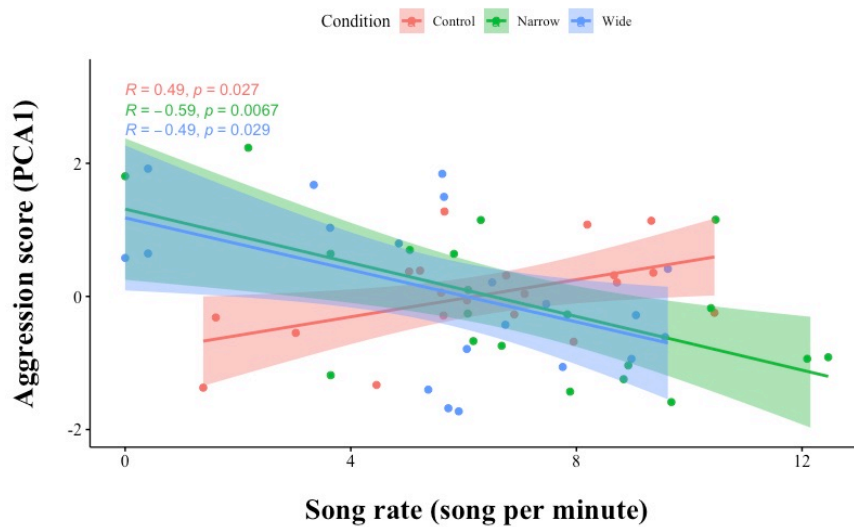


Figure 3.5: Scatter plot showing the correlations (Pearson correlation) between the aggression score (PC1) and song rate (song per minute) with respect to treatment (n=20). There are three observations for each subject with respect to treatment (control, narrow, wide), and each data point indicates a subject in the specific treatment group.

## CHAPTER 4

### DISCUSSION

#### 4.1 Summary

The present study aims to evaluate whether the black tie of great tits acts as a status signal in territorial interactions in the first encounter of males. Using a manipulation of black tie size of 3D model birds, we found no difference between aggression levels of males against small and large tie area opponents. Furthermore, birds also responded with similar levels of aggression towards the gray model as they did towards the models painted like great tits. We therefore could not find evidence for black tie being a signal during territorial interactions in the vicinity of nest or in territory center. The results also have implications for the use of 3D models in behavioral experiments.

#### 4.2 No Effect of Treatment on Aggression

There might be several routes to explain that there is no effect of treatment (wide vs narrow) on aggression. The first is the influence of song playbacks on the response. Songbirds rely on vocal signals to mark their territory and communicate with both neighbors and intruders (see [Searcy and Beecher, 2009]). It is well known that bird song is enough to deter intrusions as well as act as a strong stimulant to simulate an intrusion for songbirds [Krebs et al., 1978; Weeden and Falls, 1959]. Considering our intrusions are coupled with 5-min long playback stimuli, one can hypothesize that vocal signals might override visual signals under this scenario. As a result, our design might fail to show that black tie can be used as a signal in these interactions. How-

ever, it is difficult to direct the attention of subjects towards 3D models in situ without a vocal stimulant, so using playback stimuli is nearly inevitable. To overcome such overstimulation, one can calibrate the duration of stimuli in a way to prohibit overstimulation. Alternatively, playback stimuli can be stopped after allowing subjects to notice and observe the models.

The second explanation for our results is related to original theory of the SSH. It was proposed that plumage variability has a function in social interactions of flocking species, but territorial species do not have such variation unless it is useful for another reason [Rohwer, 1975]. However, there are species such as great tits that live in flocks in winter and territorial during breeding. Therefore, to the SSH, great tits are an interesting study subject since black tie is a signal of social status during flocking period. As they are territorial during breeding, this brings about a mystery regarding whether plumage ornaments that are shown to be status badges in winter flocks (i.e., black tie) retain their function during territoriality.

Our results showed that there is no general difference in aggression towards wide and narrow tie models with the tested sample. When we assume that models are perceived as expected, we can suggest that territoriality might override status and dominance hierarchy. In other words, individuals might not accept large tie competitors as superior. As a result, intruder's tie area might not alter the aggressive behavior of territory owners.

Previous studies examining the badge of status in great tits were mainly focused on competition over food in wintering flocks (see [Järvi and Bakken, 1984, Järvi et al., 1987, Wilson, 1992]). The present study, on the contrary, focuses on the conflicts by simulating territorial intrusions in which the interactions can be interpreted as a competition for mate, paternity, nest site or a territory itself rather than a competition over a trivial resource like food. Our observation of no general difference in aggression with respect to treatment might be a result of the great value associated with these resources because the value of the resource creates a notable asymmetry in establishing dominance, and it has a significant role in status signaling as status badges are proposed to function in resolving conflicts when the resource value is low [Maynard Smith and Harper, 1988, Rohwer, 1975]. Within our framework, one can expect



that individuals attribute an immense value to the resource, and this might alter the dominance hierarchy since they tend to venture higher costs to maintain or obtain the resource [Popp, 1987]. As a result, an opponent with a wider tie cannot dominate an individual that it used to dominate in winter where resources can be comparatively compensable.

The eagerness of the residents to preserve status quo emerges from their elevated motivation [Maynard Smith and Harper, 1988] and occasions where tie size failed to predict the outcome of conflicts supports this view [Senar, 1999]. Evidence from the previous research is in line with our findings and shows that tie size does not determine dominance hierarchy in the presence of a motivational asymmetry such as site dominance and prior residency, proximity to breeding territory, prior experience, female presence, and hunger level [Andersson and Åhlund, 1991, Balph et al., 1979, De Laet, 1984, Lemel and Wallin, 1993, Sandell and Smith, 1991, Wilson, 1992]. Thus, the reason for observing no difference in aggression levels under narrow and wide tie treatments could be explained by the elevated motivation of territory holders to maintain the resource and the elevated testosterone levels [Rohss and Silverin, 1983, Van Duyse et al., 2003] support this.

It was hypothesized that competition becomes more severe as the resources become more appealing [Maynard Smith and Harper, 1988]. From another aspect, these resources can be regarded as more costly to lose, and this could be the reason of the elevated motivation. Likewise, this motivational is associated enhancement to more damage to owners in case they lose [Rohwer, 1982]. In support of this, more intense contests on the occasions where prior resident great tits were defeated was observed [Sandell and Smith, 1991]. Overall, the black tie of great tits can function as a signal but motivational condition of competitors and value of the resource affect its function.

In a study on dark-eyed juncos, a significant seasonal increase in agonistic encounters was observed from midwinter to early spring [Balph, 1977]. Moreover, there was a significant correlation between rank and aggression in males of dark-eyed juncos during winter. However, this correlation was disturbed as the season shifted towards spring, and it was proposed that there might be a greater seasonal escalation in aggres-

siveness of subordinates compared to dominants [Balph, 1977]. A similar situation may be valid for great tits as well. Circannual circulation of testosterone shows a peak in breeding season [Rohss and Silverin, 1983, Van Duyse et al., 2003]. Testosterone is related to territoriality and aggression [Wingfield et al., 1987], and it is costly for individuals to secrete more testosterone [Wingfield et al., 2001]. Despite this cost, co-occurrence of annual peak of testosterone and breeding seems to be necessary for nest defense and overcoming intruders particularly when intruders are higher in fighting ability and resource is costly. Besides, the cost associated with the testosterone secretion might be compensated by superior benefits. These benefits are very likely to be the prevention of extra pair paternity (EPP) and defense of the nest site in case of intruders of any status. Under these circumstances, physiological changes that are associated with territoriality and breeding might override any differences in behavior that may correlate with plumage ornaments. Thus, badge size might not correlate with aggression or status.

Another explanation about the role of black tie in territorial interactions is based on the proximity to the nest and territory center. Black tie might be a signal in territorial interactions but might not function when confrontations are held in the close vicinity of nests. As the distance from nest increases, black tie might be an efficient tool to settle conflicts. This would eventually prove that the value of the resource is important within this context. In addition to this, the study period is an important variable because as the time passes after the claim of a territory by an individual, its value increases and it becomes harder to outcompete owners [Sandell and Smith, 1991, Krebs, 1982]. Our study period coincided with nest preparation and egg-laying periods and these periods cannot be considered as early. Nevertheless, we witnessed a vast number of interactions occurred between males and couples during our trials and these ranges in proximity to the nest site and territory center. However, one can expect that black tie might be a signal prior to territory establishment in which individuals have not assessed a great value to territories yet.

### 4.3 STIs with 3D Models

The use of dummies in avian ecology is a traditional approach and this contributes to the authenticity of intrusion. The traditional way of dummy production is the use of stuffed animals. 3D printing, on the other hand, is a more innovative alternative and it offers many opportunities to researchers in ecology and evolution [Behm et al., 2018, Iqic et al., 2015, Walker and Humphries, 2019]. For instance, printing 3D models instead of taxidermic models, has the potential to increase sample size, reduce pseudoreplication (due to multiple uses of the same taxidermic model) and standardize visual stimuli. Besides, 3D models can be easily redesigned and reused simply by painting. This technology requires lower expenditures compared to traditional methods with an excellent opportunity to customize models according to various design, and 3D models can be equally successful as taxidermic mounts [Natola and Toussley, ]. The easiness of customization allows researchers to modify models according to necessity of their research and design. This is particularly important and convenient for comparative designs in which researchers change only one variable (i.e., tie area, hood darkness). The accuracy of the 3D models, particularly in studies of visual signaling, however, needs to be established as well.

In our study, we tested the accuracy of the 3D model with a control condition (Figure 2.3). We expected that subject's aggressiveness would be different towards control model compared to narrow and wide tie models. Surprisingly, we failed to observe a significant difference in overall aggressive responses to models painted as a great tit (narrow & wide) and a monochrome gray model (control). This latter finding suggests caution in the interpretation of the results and underlines the need to include controls in the experimental design with 3D models.

However, the association between the vocal behavior and aggression was modified by the model: like in the previous studies [Akçay et al., 2020, Hutfluss et al., 2021], we found a negative correlation between song rate and aggression in the two great tit model conditions (Figure 3.5). But in the gray model treatment, there was a positive correlation between song rate and aggression (Figure 3.5). From this perspective, this positive correlation in control condition might potentially indicate that subjects perceived the control model differently and control condition worked efficiently.

#### 4.4 Order effect

We observed a decrease in responsiveness of subjects as trials proceed (Figure 3.2). Trials were conducted in a consecutive order even though several delays occurred. We suspect that respondent fatigue and familiarity gained due to consecutive day trials caused the decrease in responsiveness with the strong impact of playback stimuli as mentioned earlier. Since our population of interest is not ringed, we had to perform trials as quick as possible. Otherwise, possible territory takeovers and potential deaths would prevent us from completing the trials of a subject. The ultimate way to overcome the above problem is to study with ringed population over a longer period. However, keeping 1 or 2 days between trials of a subject might help while working with non-ringed populations as well.

We aimed to have a counterbalanced design by the random assignment of the sequence of models and trials so that we could have decrease the effects of limitations like order effect or habituation. Due to the moderate sample size, we did not end up with a totally counterbalanced design, but we believe it lessens the effects of trial order to some extent (see Table H.1 in Appendix H).

#### 4.5 Tie Area Measurements

We were unable to measure the tie area of subject birds. This would have allowed us to better conceptualize the relationship between tie area and status. To compensate for this deficiency, we relied on the lower and upper boundaries of the tie area variation (see Riyahi et al., 2014) of great tits in modeling 3D models. Hereby, we believe that we generally kept the target's tie area in the interval of 3D models' tie area. Therefore, our 3D models were representing least subordinate (narrow tie model) and most dominant (wide tie model) individuals (Figure 2.3).

## 4.6 Moderate Sample Size and Study Population

It is important to note that this research was conducted with a moderate sample size ( $n = 20$ ), and the decrease in half of the subject's (10 of 20) aggression scores passing from wide to narrow model is intriguing. To reach a power of 0.80 (for Cohen's  $d = 0.8$ , large effect size), at least 60 subjects are needed in our design (see Table 4.1). Moreover, these birds are from the same population, and it is likely to have genetically related individuals in experiments and analysis.

Table 4.1: Power analysis results for Cohen's  $d = 0.8$  (large effect size) and power of 0.80.

Sample size	Cohen's $d$	Power
20	0.8	0.31
60	0.8	0.79

## 4.7 Conclusion

In conclusion, we failed to show any evidence for black tie to be a signal in territorial interactions. Nevertheless, we believe that our study has yielded important methodological outcomes to improve the further work on great tits and other species using 3D models and STIs. The study of status signaling might require a comprehensive approach to better conceptualize the role of ornaments in communication and to resolve the contradictions about ornament's function. It is ideal to work with ringed populations in which several key information such as tie size, age, and physical measures (i.e., wing length, tarsus length) were already known. Furthermore, it would be helpful to perform STIs in different regions of subject's territories. With the aid of a distance gradient (i.e., distance from the nest), the relationship between resource value and functionality of status badges can be further conceptualized.



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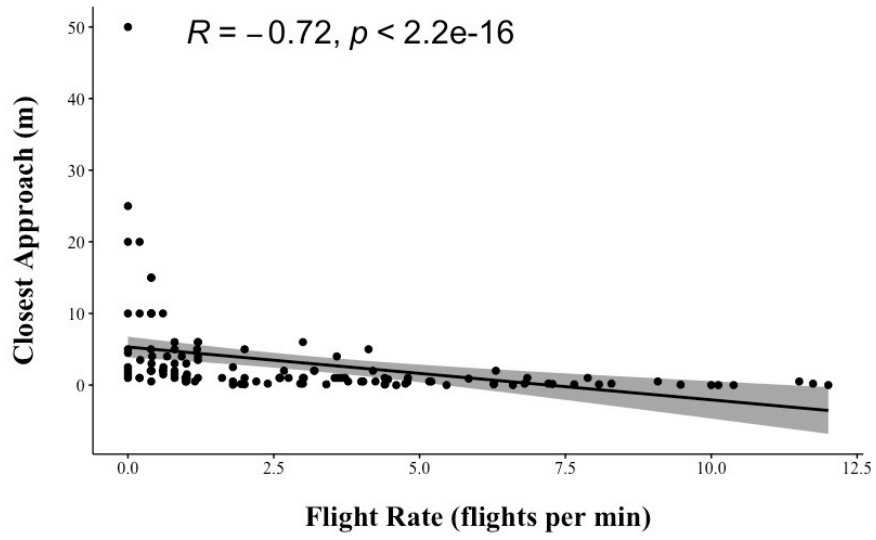


Figure A.2: Scatter plot showing the correlation between closest approach (m) and flight rate (flights per min).

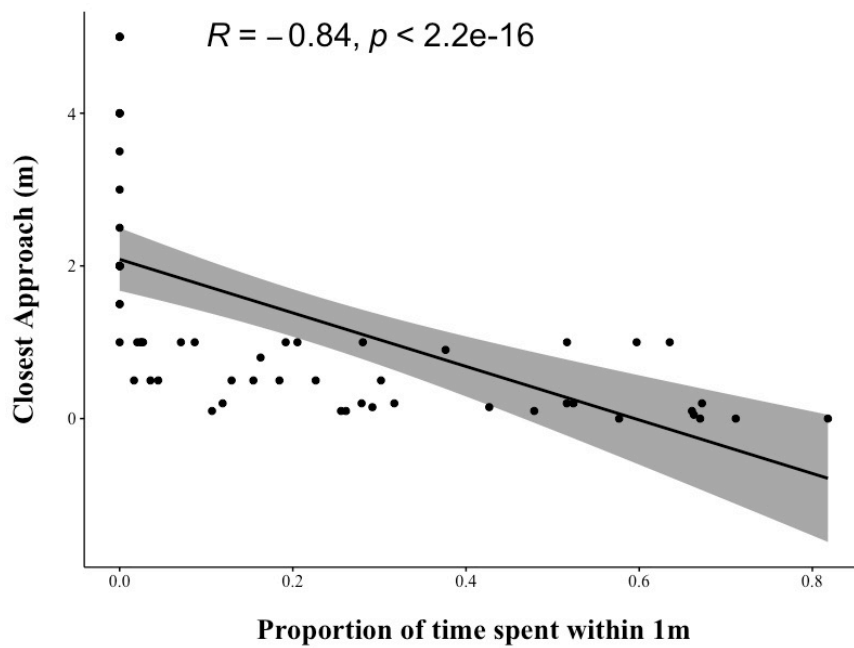


Figure A.3: Scatter plot showing the correlation between closest approach (m) and proportion of time spent within 1m of the model.



## Appendix B

### CORRELATIONS BETWEEN AGGRESSION SCORES (PCA1) AND AGGRESSION MEASURES.

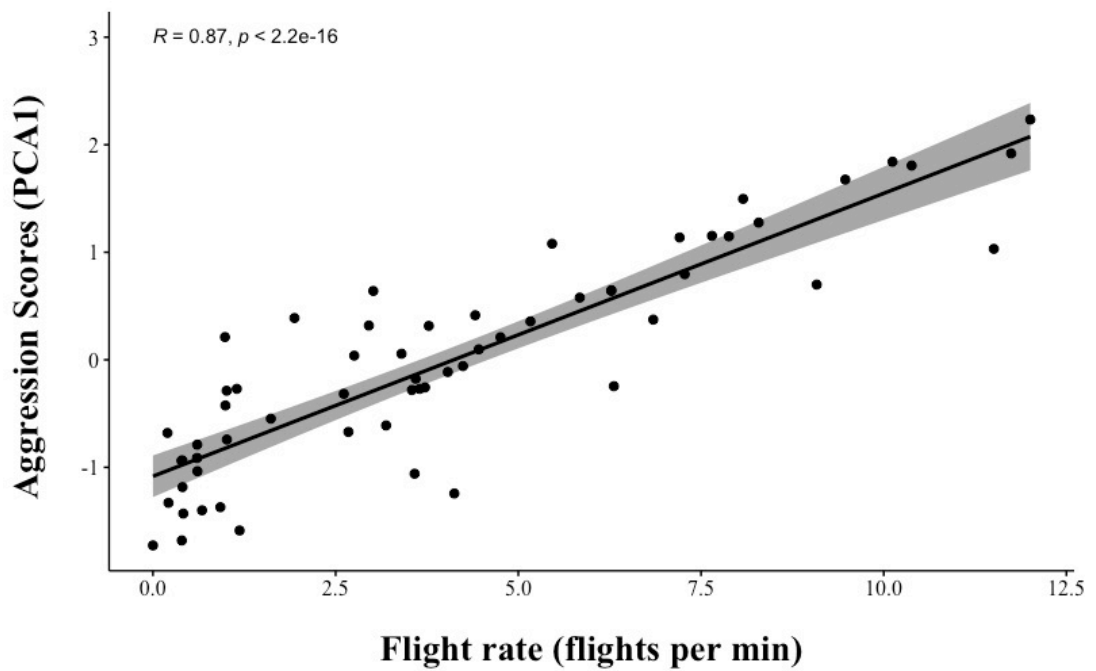


Figure B.1: Scatter plot showing the correlation between aggression scores (PCA1) and flight rate (flights per min).

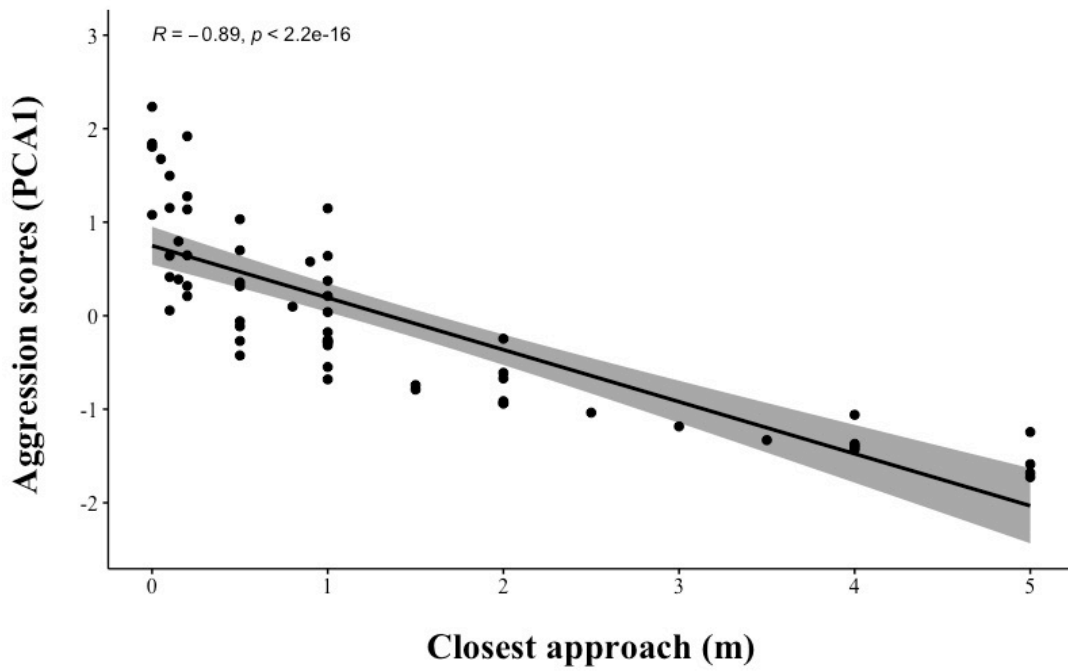


Figure B.2: Scatter plot showing the correlation between aggression scores (PCA1) and closest approach (m).

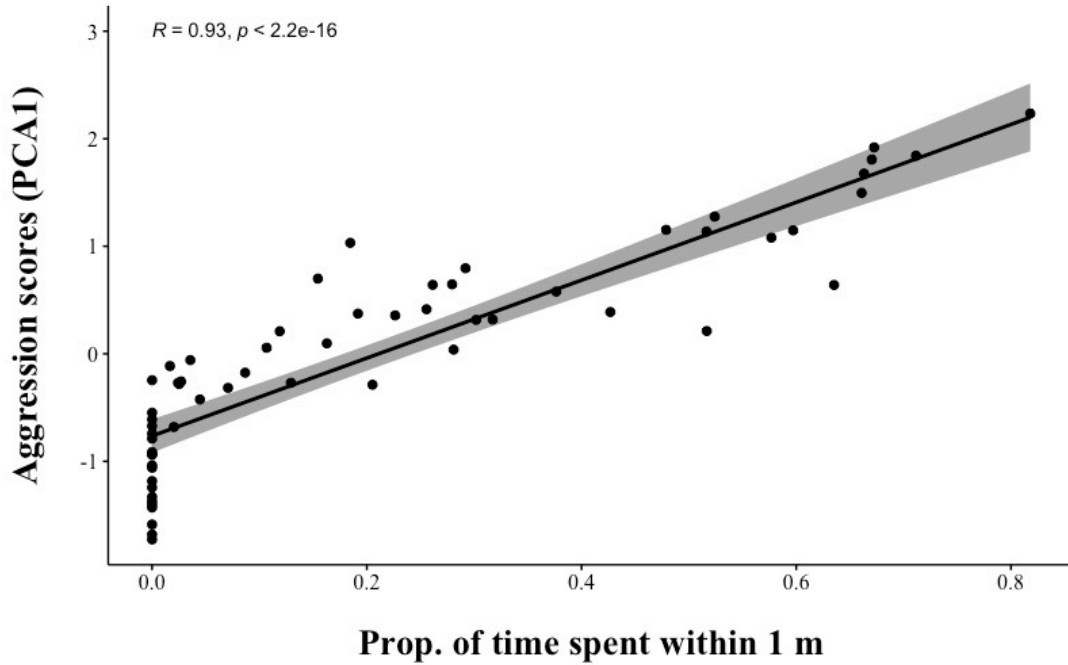


Figure B.3: Scatter plot showing the correlation between aggression scores (PCA1) and prop. of time spent within 1 m of the model.

## Appendix C

### LMM TABLE OF AGGRESSION SCORES (RESPONSE) WITH TRIAL ORDER (FIXED).

Table C.1: Linear mixed model with aggression scores as response measure and trial order is the fixed factor. Subject ID was used as random factor.

	<b>Beta estimate</b>	<b>Std. Error</b>	<b>Df</b>	<b>t-value</b>	<b>p-value</b>
<b>Intercept (Trial 1)</b>	0.34	0.22	38	1.53	0.13
<b>Trial 2</b>	-0.35	0.22	38	-1.57	0.12
<b>Trial 3</b>	-0.65	0.22	38	-2.94	0.0056 *

\* $P < 0.05$



## Appendix D

### LMM TABLE OF AGGRESSION SCORES (RESPONSE) WITH TREATMENT AND TRIAL ORDER (FIXED).

Table D.1: Linear mixed model with aggression scores as response measures with treatment and trial order as the fixed factors. Subject ID was used as random factor.

	<b>Beta Estimate</b>	<b>Std. Error</b>	<b>Df</b>	<b>t-value</b>	<b>p-value</b>
<b>Intercept (Trial 1-Control)</b>	0.38	0.26	36	1.48	0.15
<b>Narrow</b>	-0.19	0.24	36	-0.78	0.44
<b>Wide</b>	0.006	0.23	36	0.02	0.98
<b>Trial 2</b>	-0.29	0.23	36	-1.25	0.22
<b>Trial 3</b>	-0.66	0.28	36	-2.92	0.006 *

\* $P < 0.05$



## Appendix E

### ANOVA TABLE FOR LMM AGGRESSION SCORES (RESPONSE) AND TREATMENT (FIXED).

Table E.1: ANOVA table (Type III tests) for LMM of aggression scores (response) with treatment (fixed).

	Wald Chisq	Df	Pr(>Chisq)
<b>Intercept</b>	0.01	1	0.92
<b>Treatment</b>	0.57	2	0.75

\* $P < 0.05$





## Appendix F

### LMM TABLE OF SONG RATE (RESPONSE) WITH TREATMENT AND TRIAL ORDER (FIXED).

Table F.1: Linear mixed model with song rate as response measure with treatment and trial as the fixed factors. Subject ID was used as random factor.

	<b>Beta Estimate</b>	<b>Std. Error</b>	<b>Df</b>	<b>t-value</b>	<b>p-value</b>
<b>Intercept (Trial 1-Control)</b>	6.86	0.80	36	8.66	<0.01
<b>Narrow</b>	0.25	0.85	36	0.29	0.77
<b>Wide</b>	-0.86	0.81	36	-1.06	0.30
<b>Trial 2</b>	0.28	0.84	36	0.33	0.74
<b>Trial 3</b>	-1.24	0.81	36	-1.53	0.14

\* $P < 0.05$



## Appendix G

### ANOVA TABLE FOR LMM SONG RATE (RESPONSE) AND TREATMENT (FIXED).

Table G.1: ANOVA table (Type III tests) for LMM of song rate (response) with treatment (fixed).

	Wald Chisq	Df	Pr(>Chisq)
<b>Intercept</b>	96.38	1	0.92
<b>Treatment</b>	2.79	2	0.25

\* $P < 0.05$



## Appendix H

### TRIAL COUNTS FOR EACH TREATMENT WITH RESPECT TO TRIAL ORDER.

Table H.1: Trial counts for each treatment with respect to trial order.

	1st trial	2nd trial	3rd trial
<b>Control</b>	7	4	9
<b>Narrow</b>	5	11	4
<b>Wide</b>	8	5	7