Sexual imprinting on a novel blue ornament in zebra finches

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Summary

Sexual imprinting plays an important role for the development of mate preferences in birds. We tested whether male and female zebra finches *Taeniopygia guttata* sexually imprinted on an evolutionary novel trait, a blue feather on the forehead in parents. Additionally, we tested whether males and females would transfer a learned preference for the blue feather to a preference for blue leg bands, and whether a potential mate with two novel blue traits can amplify its attractiveness. Offspring were raised by parents in four different imprinting groups: (1) both parents unadorned; (2) the mother adorned only; (3) the father adorned only; (4) both parents adorned with the blue feather. After young reached maturity, we tested their mate preference for adorned and unadorned conspecifics of the opposite sex in binary choice tests. Females of the father adorned imprinting group sexually imprinted on the blue feather, and females reared by adorned parents showed a tendency to prefer adorned males. None of the males sexually imprinted on the blue feather. Our study replicates the results of a previous study on sexual imprinting on a red feather in zebra finches. We, therefore, propose a sex difference in the learning process of sexual imprinting. Females sexually imprinted on the blue feather did not transfer the learned preference to males with blue leg bands, and two novel blue traits could not amplify the attractiveness in males and females for the opposite sex. Our study emphasizes the role of sexual imprinting for the cultural evolution in mate preferences in zebra finches.

Keywords: sexual imprinting, zebra finch, novel ornamentation, mate choice.

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Introduction

Sexual imprinting is one of the important social learning mechanisms, which can determine mate preferences and a powerful mechanism for the cultural transmission of mate preferences between generations (ten Cate & Vos, 1999; Witte & Sawka, 2003). Sexual imprinting is the result of a complex learning process in which young individuals learn specific traits of their genetic or social parents during early development, and this learning process influences mate preferences later on in life. Due to sexual imprinting offspring prefer conspecifics as mates, which resemble their parents in those specific traits. Sexual imprinting has been extensively investigated in birds (Bolhuis, 1991; ten Cate & Vos, 1999), and has been shown to be important for forming mate preference in mammals (Kendrick et al., 1998; Penn & Potts, 1998; Owens et al., 1999) including humans (Bereczkei et al., 2004).

Bischof (1994) proposed a two-step process for sexual imprinting in zebra finches. In the first phase, the acquisition phase, young learn stimuli of their environment and characters of their (foster)-parents and form representations of the environment and social parents. During the second phase, the consolidation phase, young birds learn by interaction with conspecifics which of the representations are possible mates. The peak of the consolidation phase co-occurs with the time of maturation in zebra finches, i.e. around days 90-95. The early acquired information is now linked to sexual preference and stabilized (Oetting et al., 1995; Oetting & Bischof, 1996). Recently, it has been shown that the process of sexual imprinting is accompanied by neural changes in specific brain areas in zebra finch males (Bischof, 2003).

Sexual imprinting is important for species recognition (Immelmann, 1972), optimal outbreeding (Bateson, 1978), and sex recognition (Immelmann, 1975; Vos, 1994). Furthermore, sexual imprinting plays an important role in sympatric speciation (Irwin & Price, 1993). Payne et al. (2000) showed experimentally that the young of the brood-parasitic Village indigobird, *Vidua chalybeata,* sexually imprinted on a new host species, the Bengalese finch, *Lonchura striata.* Young indigobirds, which were raised by the new host species preferred mates, which has been raised by the same new host as well. Thus, within the vidua finches a new branch has been established within one generation, which is the first step for speciation. Such a host switch in indigobirds has now been discovered in nature. A wild population of Village indigobirds has recently switched to a new host, the brown firefinch, *Lagonosticta nitidula* (Payne et al., 2002). This population forms a distinct breeding population and coexists with other Village indigobirds, which parasite on the usual host, the red-billed firefinch, *L. senegala* (Payne et al., 2002). Thus, specialization on a specific host by sexual imprinting facilitates sympatric speciation (Sorenson et al., 2003).

Sexual imprinting might also be a meaningful mechanism for the cultural inheritance of new mate preferences, and theoretical models support this idea (Laland, 1994; Aoki et al., 2001). A few studies have investigated experimentally the role of sexual imprinting for the cultural evolution of novel mate preferences within a species (ten Cate & Bateson, 1988, 1989). Witte et al. (2000) introduced an evolutionary novel ornament, a red feather on the forehead, in the monomorphic Javanese Mannikin, *Lonchura leucogastroides*. They showed that males and females sexually imprinted on this novel trait and preferred adorned mates over naturally unadorned ones. Female Javanese Mannikins, which had sexually imprinted on the red feather, transferred this learned preference to another novel red trait, red stripes at the tail in this species (Plenge et al., 2000).

In a recent study, Witte & Sawka (2003) showed that in the dimorphic zebra finch, *Taeniopygia guttata castanotis,* females but not males sexually imprinted on a red feather on the forehead. They performed four imprinting groups, in which parents reared their offspring: in one group both parents were adorned with the red feather, in a second group the father only was adorned, in a third group the mother only was adorned and in the fourth group both parents were unadorned. They found that females reared by an adorned father or adorned parents sexually imprinted on the red feather and preferred males adorned with such a red feather in binary choice tests after females had reached maturity. Surprisingly, none of the males sexually imprinted on the red feather.

Although the feather on the forehead, standing upright and mimicking a crest, was an evolutionary novel trait in zebra finches, the colour of the feather was not a novel stimulus. Males have red bills and females prefer males with a redder bill (Burley & Coopersmith, 1987), and with red leg bands (Burley et al., 1982). Thus, a possible explanation why females sexually imprinted on the red feather was that females have already a bias for the colour red in mate preference due to a genetic linkage between the male trait, the red bill, and female preference for that trait (Lande, 1981; Kirkpatrick & Ryan, 1991; Bakker, 1993). Sexual imprinting on a red feather was eased in females by an already existing bias for red in male traits evolved in the context of mate choice. Interestingly, males did not sexually imprint on the red feather in the previous study. This was probably because the colour red is a male-specific trait in zebra finches, and females with a red feather look like a male and were, therefore, a negative stimulus to males (Weary et al., 1993).

To exclude those possible explanations for sex differences in sexual imprinting in zebra finches and to get a more general view and a better understanding of how sexual imprinting might support the evolution of novel traits, we repeated the study of Witte & Sawka (2003) and introduced a blue feather on the forehead, which is evolutionary novel in position, shape and colour for this species (Serle et al., 1977; Goodwin, 1982; Wolters, 1982). So far, the study by Witte & Sawka (2003) is the only one showing that male and female zebra finches differ in sexual imprinting on a novel trait. To get a better idea of whether this study shows a general pattern of sex differences in sexual imprinting it is important to repeat this study with a novel trait of a different colour. Thus, we tested in the present study whether females and males sexually imprinted on the blue feather. We did not only repeat the study by Witte & Sawka (2003), we added two novel aspects as well. We tested whether females and males transfer a learned preference for a blue feather to a preference for mates bearing another novel blue trait. Transference is a well-known phenomenon in learning processes and supports the learning process or the recognition of novel stimuli by interaction with former learning. A reaction due to transference occurs if new stimuli include characters, which are similar or identical with a character of a known stimulus. To test for transference of a preference, we introduced dark blue leg bands as another novel trait in zebra finches. These blue leg bands were of similar colour as the blue feather, and these dark blue coloured leg bands have not been used – to our knowledge – in mate choice experiments in zebra finches before.

Furthermore, we tested whether a potential mate can increase its attractiveness to a conspecific of the opposite sex when bearing two novel blue traits, the blue feather and dark blue leg bands in comparison to a bird bearing the blue feather only. So far, studies regarding sexual imprinting on a novel trait have focused on mate preferences regarding that one specific novel trait, which was introduced into the parental generation. In many species, however, males display multiple traits to females, and females base their choice on these traits (Hasson, 1989; Møller & Pomiankowski, 1993; Rowe, 1999).

Thus, we tested the role of multiple traits in male and female mate choice in zebra finches, with one novel, probably learned trait, the blue feather, in combination with a non-learned trait, dark blue leg bands.

In summary, we investigated three questions. First, we tested whether male and female zebra finches sexually imprinted on the blue feather. Second, we tested whether birds that had sexually imprinted on the blue feather transferred the learned preference to potential mates with another novel blue trait, i.e. dark blue leg bands. Third, we tested whether the attractiveness of a potential mate can be amplified when bearing both novel traits, the blue feather and dark blue leg bands, in comparison to a conspecific wearing the blue feather only.

Material and methods

Our study is a parallel study to Witte & Sawka (2003), thus, we used the same method as in the previous study. For a detailed description of the experimental set-up please look at Witte & Sawka (2003).

Imprinting groups

We randomly selected males and females of the F_3 generation of our main stock population of zebra finches. None of these birds were offspring produced in the study by Witte & Sawka (2003). We removed coloured leg bands from parents to prevent any sexual imprinting on the leg bands by the offspring. We formed four imprinting groups and assigned pairs randomly to one of them: (1) parents unadorned: none of the parents were adorned; (2) mother adorned: the mother only was adorned with a blue feather on the forehead; (3) father adorned: the father only was adorned with a blue feather on the forehead and (4) parents adorned: both parents were adorned with a blue feather on the forehead. In total we had five different pairs for the parents unadorned group, six different parental pairs in the mother adorned group, four different parental pairs in the father adorned group, and six different parental pairs in the parents adorned group. All pairs reproduced successfully.

The source of all blue feathers was a blue feather boa made of natural feathers. We cut from this boa feathers 2 cm long, elliptical in shape and with a maximum width of 0.5 cm. All feathers used in imprinting groups were identical in length and shape. The base of the feather was glued with a small piece of double-sided tape on natural forehead feathers of the birds, so that the blue feather stood upright like a crest.

We adorned parents in three imprinting groups when chicks were 8 ± 1 days old, so one or two days before the chicks open their eyes. The sensitive phase for visual stimuli starts when the young open their eyes (Immelmann, 1972). We controlled the adornment daily and reglued the blue feather if necessary. On day 20, we marked the young with orange-coloured leg bands with numbers for identification; orange leg bands are assumed to be neutral in mate choice in zebra finches (Burley et al., 1982).

When the young were 60 days old, we separated them from their parents. We kept all young from the same imprinting group together in a separate aviary. These four aviaries were visually isolated and contained food, water and sand ad libitum as well as perches. To each group we introduced an adult male and an adult female, which were ornamented in the same manner as the parents of the young. We presented these adult birds to the young because experiences later on in development can also influence mate preferences (ten Cate et al., 1984; Bischof & Clayton, 1991; Domjan, 1992; Bischof, 1994; Oetting & Bischof, 1996). This experimental condition simulated a situation in the field where young and non-reproducing zebra finches live in groups (Zann, 1996). We introduced only one adult pair to each aviary, because we wanted to simulate a rather conservative situation, in which a novel phenotype is still rare in a population. When young birds were 100 days old or older, we tested their sexual preference for adorned versus unadorned conspecifics of the opposite sex.

Mate choice tests

We performed all mate choice tests in cages (49 × 43 cm and 50 cm high) under lights with daylight spectrum. We performed tests daily between 1000 hours and 1700 hours between December 2000 and July 2001. The zebra finch is a biparental species, so we assumed that both sexes are choosy (Zann, 1996), and we performed female and male mate choice tests.

Two cages with one stimulus bird each stood side by side in front of a test bird's cage (Figure 1). Each cage contained water, seeds and sand ad libitum and four perches: one low perch near and parallel to the front to make the food more accessible, two perches of intermediate height parallel to the

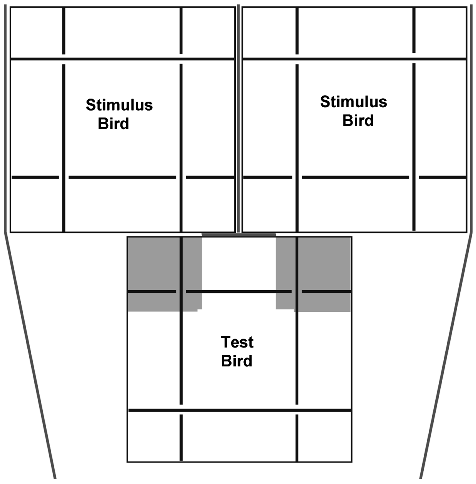


Figure 1. Top view of the binary choice situation in female and male mate choice tests. Two cages for stimulus birds stood side by side with a wooden partition in between to prevent visual communication between stimulus birds. The cage for the test bird stood in front of both stimulus birds' cages. Grey area indicates preference zones in the test bird's cage.

sides of the cage (perches of choice) and one high perch near and parallel to the back. A wooden partition between the stimulus birds' cages excluded visual contact between stimulus birds, and other screens beside and behind the cages excluded other visual stimuli. All birds were kept in test cages at least 1 day to acclimate before testing in visual but not acoustic isolation. Before the test started, the cage of the test bird was hidden behind another screen in front of the cages of the stimulus birds. Immediately after removing this screen, we began the test, which lasted 40 min, with a break at 20 min to switch the position of the two cages of the stimulus birds to control for side preference in test birds (see below). A 15-cm-wide baffle, vertically attached to the front of the test bird's cage, prevented the test bird from seeing the right stimulus bird when perching adjacent to the left stimulus bird and vice versa.

We measured the attractiveness of stimulus birds as the time (s) that the test bird spent perching on the outer one-third of the perches of choice adjacent to the stimulus bird when facing them or when clinging to the mesh above these perches in front of them (grey area in Figure 1). Other positions were scored as no-choice positions, even when the test bird was near a stimulus bird's cage but below the perches, because it was not clear whether the test bird was interested in the bird or in the food. During tests we recorded the position of the test bird every 5 s. Perch time was scored as 2.5 s when the test bird changed perches within an 5 s-interval; otherwise it was scored as 5 s.

Test males and females that showed side preferences were excluded from the analysis. Test birds showed side preference when they spent more than 80% of the time they spent in both preference zones on the same perch of choice, even though we switched the position of the stimulus cages.

In male mate choice tests, we measured the time that a test male spent in front of females and the number of courtship displays to each stimulus female as a measure of female's attractiveness to males.

To test whether the feather manipulation or wearing blue leg bands (see below) had an influence on males' behaviour we measured the number of non-directed song bouts of stimulus males in female mate choice tests. Non-directed song bouts of males play no role in female mate choice in zebra finches. Non-directed song bouts can be used as a measure of general activity in males and can show whether the manipulation has a negative influence on male behaviour or not.

Validation of the sampling method

In ten randomly chosen female choice tests, we tested how well the time we measured with our sampling method represented the real time a female perched in front of the two males. In these ten experiments, we placed a video camera (Panasonic NV-M5) one meter in front of the three cages and recorded the behaviour and positions of the birds. Afterwards we played the videos back on a TV monitor (SABA M5520C, 50 Hz), which was connected to a JVC S-VHS video recorder (HR-S 7500 E/EH) and measured the time a female spent perching in the preference zones in front of males with two stop watches. The time measured with our instantaneous sampling method correlated well with the real time measured from video analysis (Spearman's rank correlation: $r = 0.997$, $N = 10$, $p = 0.001$). Thus, our measurement of time is a very good estimate of the real time females perched in front of stimulus birds.

We performed three different experiments. In experiment 1, females and males could choose between two opposite sex stimulus birds, one with the blue feather and one without the blue feather on the forehead. Here, we tested whether females and males had sexually imprinted on the blue feather. The unadorned bird had a piece of glue on the forehead covered with natural grey forehead feathers, so that unadorned birds were manipulated in the same manner as adorned ones. In experiment 2, females and males could choose between two opposite sex stimulus birds, one with blue leg bands and one without blue leg bands. This should show whether those birds, which had sexually imprinted on the blue feather would transfer the new preference to potential mates with blue leg bands. In experiment 3, females and males could choose between two opposite sex stimulus birds, one adorned with a blue feather and one adorned with a blue feather and blue leg bands. Here, we tested whether adding blue leg bands can amplify the attractiveness of a mate with the blue feather.

We measured the relative reflectance of the blue feather and blue leg bands using an AvaSpec-2048 Spectrometer with an AvaLight-DHS Deuterium-Halogen light source (both AVANTES) covering a range between 300-700 nm (Figure 2). The blue feather and the blue leg band had a similar reflectance pattern, although the blue leg band shows a higher reflectance, probably due to a glossier surface (Figure 2).

In total, we tested 36 males and 35 females raised in the four conditions and used a maximum of two siblings of the same sex of each imprinting group (parents adorned: 9 males and 9 females; father adorned: 8 males and 8 females; mother adorned: 10 males and 8 females; and parents unadorned: 9 males and 10 females).

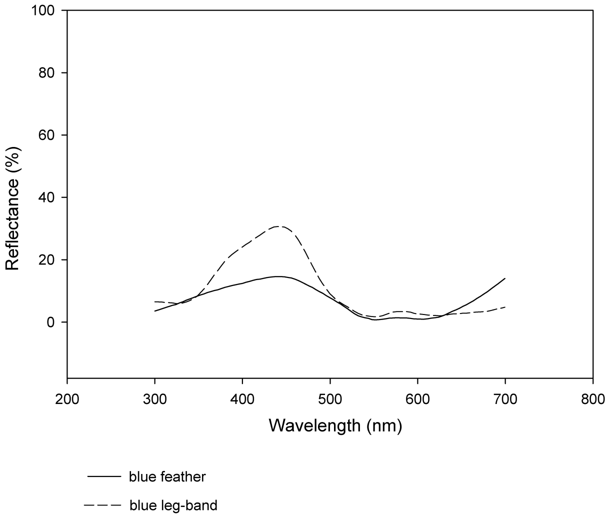


Figure 2. Reflectance curves of a blue feather and a blue leg band.

Each bird was tested in all three experiments. The sequence of the experiments was randomised across subjects. Each bird was tested in front of different stimulus birds in the three experiments. We used in total 108 stimulus males in female mate choice tests, and 108 stimulus females in male mate choice tests. Stimulus birds were used twice, but never a second time together with the same stimulus bird, and test birds were tested in front of six different stimulus birds in the three experiments.

Analysis

With a Kruskal-Wallis test, we compared the motivation of test birds of the four imprinting groups to perch within the preference zone in front of stimulus birds. The motivation is the time a test bird spent in front of both stimulus birds within a test. We used a linear mixed model with imprinting group as a fixed factor, song activity of adorned males as a covariate and relatedness and the individual as a random factor. Because relatedness had no effect on mate preferences, neither in females (linear mixed model: $F_{1,14} = 1.646$, $p = 0.179$) nor in males (linear mixed model: $F_{1,14} = 1.433$, $p = 0.260$) we removed this random factor from the final model. We used SPSS version 13 for analysis. We applied a ln-transformation, so that the data were not significantly different from a normal distribution (Shapiro Wilk's test: $0.17 < p < 0.88$) and variances were homogenous. We used a LSD test for multiple comparisons as a post hoc test. All p-values are two tailed. To test whether the manipulation (feather or leg band) had any influence on male behaviour we compared the number of non-directed song bouts of stimulus males over all female mate choice tests with a Wilcoxon signed-rank test. In male mate choice tests, we compared the number of courtship displays to an adorned or double-adorned female with a Kruskal-Wallis test.

Results

Female mate choice tests

Blue feather

We excluded two out of 35 females from the analysis because of side preferences: one female from the mother adorned group, one female from the parents adorned group. We compared the responses of females from all four imprinting groups to males adorned with the blue feather. The imprinting group had an effect on female mate choice (linear mixed model: $F_{3,28} = 3.283$, $p = 0.035$, Figure 3a). The song activity of adorned males had no influence on female mate choice (linear mixed model: $F_{1,28} = 0.138$, $p = 0.713$). A post hoc test for multiple comparisons showed that the significant differences were due to differences between the father adorned and the parents unadorned group (LSD pairwise test: $p = 0.011$, Figure 3a) and between the father adorned group and the mother adorned group (LSD pairwise test: $p = 0.006$, Figure 3a). Thus, females of the father adorned group showed a strong preference for males adorned with a blue feather and sexually imprinted on the blue feather. Although we found that only females of the father adorned group spent significantly more time with adorned males than females of the other three imprinting groups, we would like to mention that females reared by adorned parents showed a tendency to prefer adorned males over unadorned males (Binomial test: $N = 8$, $p = 0.07$).

Singing activity in males

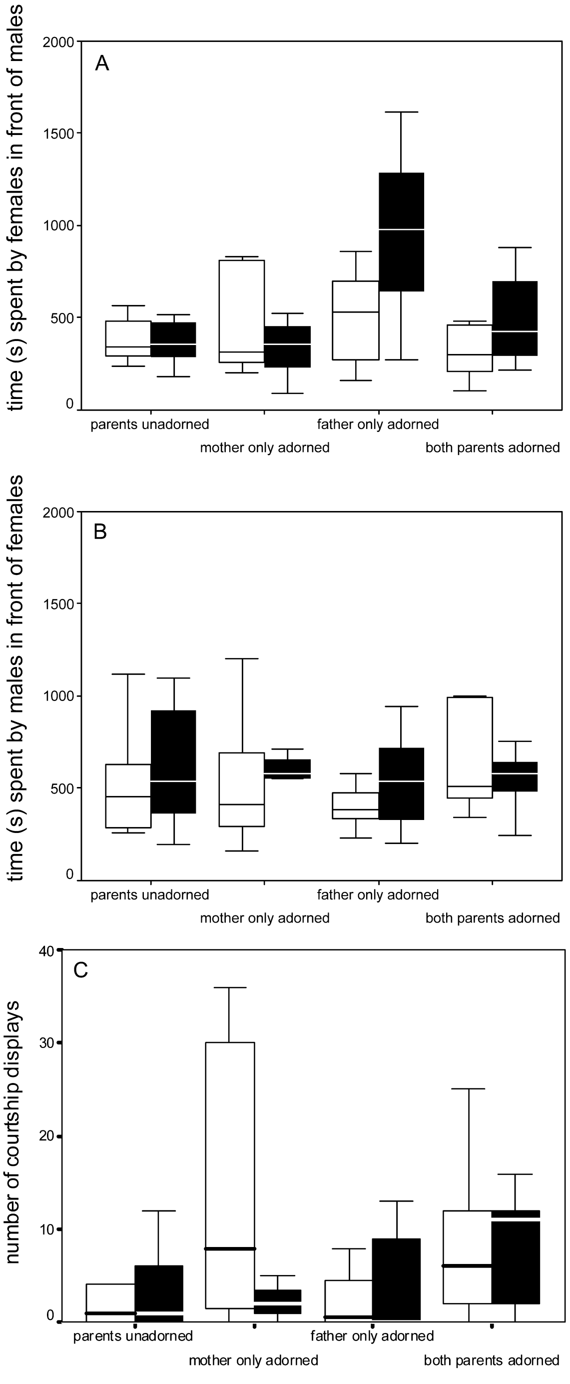
To test whether the feather manipulation influenced the song activity of males adorned with the ornament, we compared the number of non-directed song bouts of males that were used twice as stimulus birds, once as an unadorned male (with the piece of glue covered with natural grey forehead feathers) and once as an adorned male (with a crest feather). Males sang slightly more often when they were adorned with the blue feather (adorned males: median 0, range 0-18, unadorned males: median 0, range 0-13) (Wilcoxon signed-ranks test: $z = 1.9$, $N = 83$, $p = 0.053$). This showed that the manipulation had no negative effect on males' singing activity.

Blue leg bands

In this experiment, the imprinting group had no effect on female mate choice (linear mixed model: $F_{3,28} = 0.749$, $p = 0.532$). In all four imprinting groups, females showed no significant preference for males adorned with dark blue leg bands.

Singing activity in males

To test whether wearing blue leg bands influenced the song activity of males, we compared the number of non-directed song bouts of the same males with blue leg bands and without leg bands. Males did not differ in their song activity (Wilcoxon signed-rank test: $T = 4$, $N = 17$, $p = 0.34$).


A
time (s) spent by females in front of males
0
500
1000
1500
2000
parents unadorned
mother only adorned
father only adorned
both parents adorned
B
time (s) spent by males in front of females
0
500
1000
1500
2000
parents unadorned
mother only adorned
father only adorned
both parents adorned
C
number of courtship displays
0
10
20
30
40
parents unadorned
mother only adorned
father only adorned
both parents adorned

Blue leg bands and a blue feather versus blue feather

We excluded two females of the parents adorned group due to side preferences. There was no effect of the imprinting group on female mate choice (linear mixed model: $F_{3,28} = 0.381$, $p = 0.767$). In all four imprinting groups, females showed no significant preference for males adorned with a blue feather only or for males bearing a blue feather and dark blue leg bands. Thus, it seems that a second blue trait did not change the attractiveness of males adorned with the blue feather to females of these four imprinting groups.

The motivation of females to perch within the preference zones was similar between females of the four imprinting groups over all tests (Kruskal-Wallis test: $H_3 = 6.45$, $N = 35$, $p = 0.092$).

Male mate choice tests

Blue feather

We excluded three males of the mother adorned group and two males of the parents adorned group due to side preferences. Our analysis revealed no significant effect of imprinting group (linear mixed model: $F_{3,28} = 0.776$, $p = 0.517$, Figure 3b). In all four imprinting groups, males showed neither a preference for adorned females nor unadorned females (Figure 3b). Males of all imprinting groups courted adorned and unadorned females equally often (Kruskal-Wallis test: $H_3 = 5.688$, $p = 0.128$, Figure 3c).

Blue leg bands

We had to exclude one male from the parents adorned group because of side preference. Imprinting groups had no effect on male mate choice (linear mixed model: $F_{3,28} = 0.483$, $p = 0.697$). Males of all four imprinting groups exhibited no preference for females bearing blue leg bands.

Between the four imprinting groups, males did not differ in courtship activity towards females adorned with blue leg and unadorned females

Figure 3. Results of mate choice tests: Box plots and whiskers show median with first and third interquartiles and minimum and maximum values for the time (a) females and (b) males spent perching in front of stimulus birds, and (c) the number of male courtship displays to stimulus birds. Stimulus birds were of the opposite sex of the test birds and were either adorned with the blue feather (■) or unadorned (□).

(Kruskal-Wallis test: $H_3 = 3.947$, $p = 0.267$). It seems that males of all four imprinting groups showed no preference for females with blue leg bands.

Blue feather and blue leg bands versus blue feather

We excluded one male from the mother adorned group and one male from the parents unadorned group due to side biases from the analysis. Our analysis revealed a non-significant effect of imprinting group on male mate choice (linear mixed model: $F_{3,28} = 0.441$, $p = 0.726$). In all four imprinting groups, males showed no preference for a female with a blue feather or a female adorned with a blue feather and dark blue leg bands. There was no significant difference in all four imprinting groups in the number of courtship displays towards females adorned with the blue feather and dark blue leg bands (Kruskal-Wallis test: $H_3 = 2.229$, $p = 0.526$). Double ornamentation seems to have no effect on attractiveness in females to males.

The motivation of males to perch within the preference zones in front of females did not differ between males of the four imprinting groups over all tests (Kruskal-Wallis test: $H_3 = 5.078$, $N = 32$, $p = 0.166$).

Discussion

Our study shows that females of the father adorned group sexually imprinted on the blue feather. Females reared by an adorned father spent significantly more time in front of males adorned with a blue feather than unadorned males. And females reared by adorned parents showed a tendency to prefer adorned over unadorned males. Females reared by an adorned mother or unadorned parents did not prefer males with a blue feather and thus, did not sexually imprint on that novel trait. In contrast to females, none of the males in any of the four imprinting groups sexually imprinted on the blue feather. Our study supports the results of the study by Witte & Sawka (2003), which found similar results: female zebra finches reared by a father adorned with a red feather on the forehead and females reared by parents adorned with a red feather sexually imprinted on a red feather. But males of the same four imprinting groups as performed in the present study did not sexually imprint on the red feather.

One criticism of the previous study was that the colour of the red feather was not a new stimulus in zebra finches because males have red bills and females prefer males with redder bills (Burley & Coopersmith, 1987). A possible explanation why females sexually imprinted on the red feather could be that females have already a bias for the colour red in mate preference due to pleiotropic effects (Lande, 1981; Kirkpatrick & Ryan, 1991; Bakker, 1993). Thus, sexual imprinting on a red feather was probably facilitated in females by an existing bias for red in male traits evolved in the context of mate choice. On the other hand, males rejected females with a red trait probably because the colour red is a male-specific trait in zebra finches, and females with a red feather look like a male and were, therefore, a negative stimulus to males (Weary et al., 1993).

The present study shows again that only females but not males sexually imprint on a novel trait. It seems, therefore, that there might be a sex difference in imprintability on a novel trait in zebra finches under these experimental conditions. To estimate whether there is a sex difference in imprintability on a novel trait, a red or a blue feather on the forehead, we used data of both studies, this study and Witte & Sawka (2003), and compared the time spent in front of adorned females by males reared by an adorned mother with the time spent in front of adorned males by females reared by an adorned father. We compared the time spent of males and females of these two imprinting groups, because, if at all, males should have learned the phenotype of the mother (adorned mother) and females have learned the phenotype of the father (adorned father). We found an almost significant sex difference in the ability to imprint on a novel trait, a red or blue feather on the forehead (linear mixed model $F_{1,36} = 4.602$, $p = 0.065$).

Why do sexes differ in imprintability on a novel trait in zebra finches under these experimental conditions? Bischof (1994) has provided a model for a two-step imprinting process on visual cues in zebra finches. The early acquisition phase starts when young birds open their eyes; that is around day 8. During the acquisition phase young learn both, stimuli in their environment and characters of their parents and form representations of the environment and social partners. The second phase, the consolidation phase, started when young birds intensively interact with conspecifics of the opposite sex and young birds learn by interaction with conspecifics which of the representations are possible mates, and the early acquired preference is linked to sexual behaviour and stabilized (Bischof, 1994). This is especially the case in males. Males approach females and perform courtship displays towards females. During the consolidation phase a presentation of a female to a male for only one hour is sufficient to stabilize the sexual preference in zebra finch males (Bischof & Rollenhagen, 1999). Females, however, rarely initiate solicitation displays towards males. This sex difference in behaviour may cause a sex difference in the learning process during sexual imprinting. The two-step learning process during sexual imprinting is assumed to be valid for female zebra finches as well, although almost all studies regarding the learning mechanism were performed with male zebra finches. On the basis of the study by Witte & Sawka (2003) and this study, we would like to suggest a modified sex-specific learning process in sexual imprinting, which considers the different behaviour of the sexes during the consolidation phase. We argue that the phase important for sexual imprinting, the phase in which young learn which of the representations are possible mates, differ in time between the sexes in zebra finches. We argue that the earlier phase is more important in females and influences mate choice later on, whereas in males, the second phase is more important for developing mate preferences and influenced mate choice decisions later on. This new interpretation of the two-step model could explain why females but not males sexually imprinted on a novel trait in our study and in the study of Witte & Sawka (2003). In both studies, females reared by an adorned father (and adorned parents, Witte & Sawka, 2003) sexually imprinted on a novel trait (blue feather and red feather respectively), although they were separated from parents on day 60 and kept in groups of young birds where all males (except of one model male) were unadorned. From day 60 onwards, females had almost exclusively experienced sexual interactions with unadorned males. Nevertheless, females exhibited a strong preference for adorned males over unadorned ones in binary choice tests. Thus, experiences due to interaction with unadorned males from day 60 onwards, did not change or override the preference for adorned males based on the experience with the adorned father, before day 60. In these females, the linkage between the learned representations and what is a potential mate occurs during the first phase of sexual imprinting. We therefore suggest that in females both phases, acquisition and consolidation phase overlap and occur during the earlier developmental phase at the same time.

Our new interpretation of the two-step process provided by Bischof (1994) can explain why males did not sexually imprint on the novel ornaments in this study and the study of Witte & Sawka (2003). From day 60 onwards, males were kept together with other young birds of the same imprinting group and all females (except the model female) were unadorned and thus, males almost exclusively interacted with unadorned females during their first courtship displays. These experiences with unadorned females formed their mate preferences, and males, therefore, did not prefer adorned females in the preference tests. Our experimental condition differs from the experimental conditions in earlier imprinting studies showing that males sexually imprinted on the foster species. In those studies, males were isolated from day 35 onwards and could interact for the first time with females at the age of 90 to 100 days. Thus, those males had no opportunity to interact with females during the consolidation phase, only in a short preference test. Our experimental condition, living in groups after independence is a more natural situation for the zebra finches than an isolation period (Zann, 1996), and we would suggest that our results therefore better reflect the situation in the wild.

We conclude from this study and the study by Witte & Sawka (2003) that there is a sex difference during the learning process in sexual imprinting. Females sexually imprint during the earlier phase, in which both learning phases overlap, while the two learning phases in males are separated by time.

Our hypothesis that females sexually imprint during the earlier phase and males sexually imprint during the second phase, can be tested with the following experiment: Females reared by a father adorned with a green feather and an unadorned mother are separated from parents on day 60 and kept in a group of males adorned with a blue feather and unadorned females. A blue and a green feather are both novel colours for the zebra finch. We would expect that females will prefer males with a green feather over those with a blue feather in binary mate choice tests because the father was adorned with a green feather, although they get experiences with males adorned with a blue feather. Males reared by a mother adorned with a green feather and an unadorned father are separated from parents on day 60 and kept together with females adorned with a blue feather and unadorned males. We would expect males to prefer females adorned with a blue feather over females adorned with a green feather in binary choice tests because males get first sexual experiences with blue adorned females and this is more important for imprinting than the interaction with the mother adorned with a green feather.

The evolutionary consequences of a sex difference during the learning process of sexual imprinting is that in a dimorphic species like the zebra finch the dimorphism is amplified by females developing a preference for a novel male phenotype whereas males did not. A functional explanation why females sexually imprint during the early phase might be that because females invest more resources into offspring it is more costly for females to mate with a male of the wrong species. Thus, when females sexually imprint on the phenotype during the early phase when they see only the parents, they are on the safer side and learn the phenotype of the proper species and of a phenotype, which may be positively correlated to fitness related qualities like parental care. Males, on the other hand, can maximise their reproductive success by copulating with as many females as possible. That is true for the biparental zebra finch as well, because males engage in mating with extra-pair mates. It might be advantageous for males to be more flexible in learning what is the right phenotype for a mate and to learn this by achieving first experiences during courtship displaying in front of females. Because females will accept or reject a courting male based on the mate preference developed by sexual imprinting, males and females will assortatively pair with a partner, which had experienced the same environment, i.e. the same parental phenotype. This has been nicely shown by Payne et al. (2000) in the brood-parasitic Village indigobird, *Vidua chalybeata.* Young indigobirds, which were raised by a new host species preferred mates, raised by the same new host as well, thus they preferred mates, which had experienced the same environment during development. Thus, a sex difference in the learning process of sexual imprinting can be explained by ultimate reasons and seems to be valid for evolutionary biology.

One question still remains, why did males not prefer unadorned females over adorned ones? We think that although the consolidation phase is more important in males than the acquisition phase, males have learned both phenotypes. And although males have mostly interacted with unadorned females they did not show a clear preference for unadorned females. They courted in front of both female phenotypes similar often (Kruskal-Wallis test: $H_3 = 5.688$, $p = 0.128$, Figure 3c), probably because they are familiar with both phenotypes and try to find a mate to maximise their reproductive success.

Blue leg bands and transference of a learned preference

Females reared by an adorned father sexually imprinted on the blue feather, but did not prefer males bearing blue leg bands. Similar was true for females reared by adorned parents. These females did not prefer males adorned with blue leg bands, although they showed a weak preference for males adorned with a blue feather. Thus, females did not transfer the learned preference for the blue feather to potential mates with another novel blue trait, blue leg bands. Transference is a well known phenomenon in learning processes and supports the learning process or the recognition of novel stimuli by interaction with former learning. A response due to transference occurs if new stimuli include characters, which are similar or identical with a character of a known stimulus. Hence, in our experiments the colour of leg bands is very similar to the colour of the feather (Figure 2), we had expected a transference of preference to males bearing blue leg bands. In the monomorphic Javanese mannikin, *Lonchura leucogastroides*, Plenge et al. (2000) showed that females that had sexually imprinted on a red feather on the forehead transferred this learned preference to males with another novel red trait, red stripes on the tail. In binary choice tests, these females exhibited a similar strong preference for males adorned with a red tail over unadorned males than for males adorned with the red feather. When comparing both studies, it seems that the learned preference for the blue feather is more specific in the dimorphic zebra finch than in the monomorphic Javanese Mannikin. In our study, we changed not only the location of the new trait by using blue leg bands, but also the shape. To get an idea whether transference in a dimorphic species like the zebra finch occurs, it would be better to test the attractiveness of potential mates with blue feathers on the tail and thus changing only one dimension.

Zebra finch males exhibited no preference for females with blue leg bands over those without leg bands. Because males did not sexually imprint on the blue feather we did not expect any preference for females with blue leg bands.

Double ornamentation – amplifying attractiveness?

In many species, males display multiple traits to females, and females base their choice on these traits. Female sedge warblers, *Acrocephalus schoenobaenus*, select males by multiple traits that reflect different aspects of male quality and territory quality (Buchanan & Catchpole, 1997). Female satin bower birds (*Ptilonorhynchus violaceus*) use bower quality and male ultraviolet plumage as cues for mate qualities (Doucet & Montgomerie, 2003). Females may base their mate choice on several different traits to have a more accurate honest indicator for male quality (Møller & Pomiankowski, 1993). Different male traits may indicate different male qualities in males and thus advertise multiple qualities (multiple message hypothesis). Or different male traits signal the same message and this is more reliable for females (back up hypothesis). Although males display multiple signals, only a few traits may provide reliable information about the condition of the male (unreliable signal hypothesis, Møller & Pomiankowski, 1993). Multiple signals may serve for signal amplification (Hasson, 1989) or for enhancing signal detection (Rowe, 1999).

Here, we asked whether a potential mate could increase its attractiveness to the opposite sex by bearing a second novel blue trait, i.e. blue leg bands. In zebra finches, coloured leg bands can have a strong effect on survival (Burley, 1985a; Zann, 1994), reproductive success (Burley, 1986a), on parental investment (Burley, 1988), on sex ratio in offspring (Burley, 1981) and on mate attractiveness (Burley et al., 1982; Burley, 1986b). Males with red leg bands were found to be more attractive to females, males with light green or light blue leg bands were found to be less attractive to females. Females with black, yellow or pink leg bands were found to be attractive to males (Burley et al., 1982; Burley, 1985b). The colour orange is neutral in both sexes (Burley et al., 1982). In our study, females reared by an adorned father which sexually imprinted on the blue feather, did not prefer males with the two blue traits, the blue feather and dark blue leg bands, over males adorned with the blue feather only. That was true for females of the other three imprinting groups as well. Thus, a second blue ornament could not increase the males' attractiveness to females.

Males of all four imprinting groups did not show any preference for either female type. Thus, a second blue trait in females did not increase their attractiveness to males. We did not expect any female type to be more attractive as male zebra finches were not sexually imprinted on a blue feather on the forehead.

To summarize, a second blue trait did not increase the attractiveness of a potential mate to our test birds, neither in male nor in female zebra finches.

In summary, our study shows that females reared by an adorned father and an unadorned mother showed a clear preference for males adorned with a blue feather and females reared by adorned parents showed a tendency to prefer adorned males. None of the males sexually imprinted on a blue feather on the forehead. Females, which sexually imprinted on the blue feather did not transfer this new preference to males bearing another novel blue trait. Individuals could not increase their attractiveness to the opposite sex by advertising two novel blue traits, the feather and blue leg bands.

This is the second study providing evidence that females but not males sexually imprinted on a novel trait in zebra finches. This leads to the question that there might be sex differences during the process of sexual imprinting. This idea should be tested in further studies.

Our study provides additional support for the role of sexual imprinting for the evolution of novel mate preferences. Sexual imprinting is an important mechanism for the cultural transmission of a new mate preference in zebra finches and might lead to an establishment of a novel trait in males. Thus, sexual imprinting would strengthen the dimorphism in zebra finches.

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